

# Good News and Bad News about DNA Fingerprinting

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IT IS SOMETIMES SAID<sup>1</sup> that new tools are largely responsible for scientific revolutions. Nothing would seem further from the truth in behavioural and evolutionary ecology, where ideas – natural, sexual, kin and group selection, optimality theory, handicap principle and sex allocation, to mention a few – have been responsible for most of the development of the discipline. There are some notable exceptions, of course: the various methods used to mark individuals for later identification are by now the *sine qua non* of behavioural ecology, and allozyme electrophoresis not only revealed an astounding level of genetic variation<sup>2</sup> but also became a useful tool for a variety of analyses in population genetics<sup>3</sup>.

The development of DNA fingerprinting<sup>4</sup> over the past six years now promises both to revolutionize our understanding of animal social systems and to alter radically the way most behavioural ecologists work. Autoradiograms (fingerprints) produced by probing DNA extracted from blood or tissue samples of adults and offspring can be used to determine an individual's parentage with a high degree of certainty<sup>4,5</sup>. While initially developed by Alec Jeffreys<sup>4</sup> to determine human parentage, the technique has now been adapted for use in studies of many other animals, particularly birds. Several recent publications, as well as presentations at the 20th International Ornithological Congress (IOC) in New Zealand last December, demonstrate clearly that this technological innovation is the basis for some important breakthroughs. While this all sounds like good news there is, regrettably, some bad news associated with this revolution.

First the good news. At the recent IOC the sense of excitement was obvious: more than ten papers used DNA fingerprinting to reveal new information about patterns of parentage, and hardly a study of mating or parental care failed to acknowledge the critical need for such analysis. For example, Lisle Gibbs (Queen's University, Ontario, Canada) and collaborators showed that male red-winged blackbirds (*Agelaius phoeniceus*) realized an average of 21% of their reproductive success in the nests of females on other males' territories<sup>6</sup>. While extra-pair copulations (EPCs) have been documented in many species<sup>7</sup>, these results suggest

that none of the extant polygyny models<sup>8</sup> can possibly apply to red-winged blackbirds, arguably the species most widely used in studies of avian polygyny.

Terry Burke (University of Leicester, UK) and collaborators showed that the distribution of fertilizations by male ruffs (*Philomachus pugnax*) at one lek did not match the distribution of copulations observed there, but that there was such a correlation in black grouse (*Tetrao tetrix*). Peter Dunn (Queen's University, Ontario, Canada) and collaborators found that tree swallows (*Tachycineta bicolor*) had much higher extra-pair fertilization (EPF) rates (more than 35% of nestlings) than observational studies of EPC rates (3% of copulations) had indicated.

In an experimental study, Henrik Smith (University of Lund, Sweden) and collaborators discovered that male barn swallows (*Hirundo rustica*) with elongated tail feathers were actually cuckolded more often than males with shortened tails<sup>9</sup>, even though previous studies had found that males with elongated tails were preferred as mates<sup>10,11</sup>, obtained more EPCs<sup>11</sup> and apparently had higher reproductive success<sup>11</sup>. Tim Birkhead (University of Sheffield, UK) and collaborators<sup>12</sup> showed that extra-pair parentage in wild zebra finches (*Taeniopygia guttata*) was due to both nest parasitism by females and EPFs by males.

In addition to revealing a new level of complexity in avian social systems, these results, as well as some recently published work<sup>13–16</sup>, demonstrate the virtual necessity of using DNA fingerprinting in any study that attempts to measure an individual's reproductive success.

Now for the bad news: DNA fingerprinting is expensive. In our own studies<sup>6,9</sup>, every dollar spent in the field requires at least another dollar spent in the fingerprinting lab, effectively doubling the costs of our research programs. In addition, the techniques used are complicated enough that specialized training of technicians is required; often, much preliminary work is needed to determine the best enzymes and probes for the production of usable fingerprints. While prices and conditions will undoubtedly vary greatly between labs and countries, we provide the following brief outline from our own

experience as a rough guide for those contemplating some fingerprinting analyses.

At the outset, the major costs are for lab facilities such as spectrophotometers, centrifuges and cold-storage equipment (US\$30 000–200 000). In addition to the general lab infrastructure, each technician also requires a workstation (US\$5000) for the preparation of DNA, Southern blotting and electrophoresis. Disposables (chemicals, gels, glassware, etc.) can cost up to US\$50–100 per individual bird sampled. These, plus technicians' salaries, bring the total cost of fingerprinting to as much as US\$100–200 per individual bird, after the lab facilities have been set up.

In our experience, a technician can learn to produce acceptable fingerprints in about two months, and can then produce usable fingerprints at a maximum rate of about 30 individuals per month after the best probes and enzymes have been found. While we believe that these are realistic estimates for an established lab, our first studies produced fingerprints at the rate of only 10–15 individuals per month. As a consequence of all this, studies requiring DNA fingerprinting will not often yield results until a year or two after field work has been completed. This accounts, in part, for the fact that so few studies employing fingerprints have appeared in the four years since the first application of this technology to a problem in behavioural ecology.

Given that DNA fingerprinting will be necessary whenever it is important to determine an individual's reproductive success, field biologists will obviously be faced with some tough decisions. The cheapest solution would be to find molecular biologists with whom to collaborate. However, there will be few molecular biologists with the means and inclination to accommodate an entirely new line of research in their laboratories, especially one that answers no new questions about DNA. An alternative is to pay a commercial lab to produce fingerprints, but this too will be costly. From the New Zealand meeting it appeared that all of these options were being pursued to some degree. Eventually, the establishment of university-based, fee-for-service labs might turn out to be the most practical solution. For some reason, possibly lack of financial

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support, no such lab has yet been established on a large scale.

The bottom line is money. Researchers must now inform their granting agencies that the costs of doing research have changed in a fundamental way. It is also time to pursue a collective strategy by establishing a few large national or international centres for fingerprinting analysis. DNA fingerprinting is becoming such an essential feature of much behavioural ecology research that it will be unfortunate if its application is limited to the few labs with both the facilities and the finances to employ this technology as a matter of course.

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## Mass Extinction of European Fungi

John Jaenike

WE USED TO think about modern-day extinction in terms of the loss of individual species: the passenger pigeon, the quagga, the California condor. In recent years, it has become apparent that extinction may affect large taxonomic groups on a regional or worldwide scale, such as the Hawaiian avifauna<sup>1</sup>, plants of tropical cloud forests<sup>2</sup>, and amphibians around the world<sup>3</sup>. As was made clear in a presentation by Eef Arnolds (Biological Station, Wijster, The Netherlands) at the 4th International Mycological Congress held in Germany last autumn, another mass extinction may be taking place right under our collective feet. In northern Europe, there has recently been a staggering decline in the abundance and diversity of ectomycorrhizal fungi, whose presence is manifest by the appearance of above-ground fruiting bodies – mushrooms.

Arnolds bases this conclusion on several lines of evidence. Perhaps the most sobering concerns the total number of species of macromycetes collected on over 8000 forays made in the state of Saarland in western Germany from 1970 to 1985. During this period, the number of species collected per year declined by nearly 60%. In the Netherlands, the average number of ectomycorrhizal fungi collected per foray remained fairly constant from 1900 through the 1960s, but started to decline significantly in the 1970s. In the 1980s, the number of such species collected per foray was

only about half of that for the first half of the century.

Intensive collecting within more restricted areas yields similar results. Sites in Germany, Austria and the Netherlands that have been sampled repeatedly reveal losses in species diversity of 40–50% over periods of 30–60 years. On replicate plots in oak forests in the Netherlands, the average number of mycorrhizal fungi declined from 37 species per plot in the early 1970s to 12 per plot in the late 1980s. The number of species of mycorrhizal fungi found in the Giant Mountains of Czechoslovakia declined by 80% between 1958 and the early 1980s.

The decline in species diversity is paralleled by equally dramatic drops in the abundance of those species that still survive. This is clearly seen in data on the quantity of mushrooms brought to market. For instance, the weight of chanterelles (*Cantharellus cibarius*) brought to the Saarbrücken market in Germany declined steadily from an average of about 6000 kg per year in the 1950s to under 200 kg in the 1970s.

Finally, the geographical ranges of many surviving species have declined substantially. Arnolds<sup>4</sup> has shown that of 21 species of hydnoaceous fungi (Basidiomycetes) native to the Netherlands, eight have not been seen since 1973 and are regarded as extinct. The number of localities in which the remaining species have been found has declined by over 90% for six of the species and between 60% and 90% for the rest.

What can be causing such a massive decline in these fungi? Harvesting by humans appears unlikely to be the culprit. In the same forests in which the chanterelles have declined so drastically, the abundance of the honey mushroom (*Armillaria mellea*), which is also collected for commercial sale, has remained unchanged. A key difference between these species is that *C. cibarius* forms mycorrhizal associations with trees, whereas *A. mellea* is parasitic on them. Furthermore, many fungal genera that have undergone the greatest declines, such as *Cortinarius*, *Amanita* and *Russula*, are of little or no economic importance.

Habitat loss may account for the decline of some species, although this seems unlikely to be a general explanation. The hydnoaceous fungi of the Netherlands, whose ranges and abundances have declined so greatly, occur in habitat types (coniferous and deciduous forests on dry sandy soil) that have actually increased in recent years<sup>5</sup>. Furthermore, drastic declines in the abundance of mushrooms have been documented on permanent forest plots that have been surveyed from the early 1970s through the late 1980s.

Arnolds argues that air pollution is the primary cause of the disappearance of ectomycorrhizal fungi, as the declines are greatest in the most heavily polluted regions of Europe. It is significant that the greatest declines in these fungi, at least in the Netherlands, have occurred in forests on nitrogen-poor soils<sup>5</sup>. Precipitation now brings an average annual input of about 60 kg of nitrogen per hectare to such forests. As D.J. Read (University of Sheffield, UK) noted in his

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