Assessing genetic diversity in wild mega animals using non-invasive methods

T. N. Khoshoo

So far the prime indicator of successful conservation of mega wild animals (both carnivores and herbivores) has been increase in their number in a given habitat. This has been aptly true of tiger. Genetic variability at population level has never been a criterion for such an assessment[1]. For instance, there are no data to show if the unique fish-, crab- and chital-eating tiger in Sunderbans (West Bengal) is genetically identical or different from the herbivore-eating tiger in Kalakad–Mundanthurai (Tamil Nadu): the last outpost of tiger in India. The two populations have been geographically isolated. The only study in India on genetic fingerprinting of the Indian tiger and lion is that of Shankaranaryanan *et al.*[2] but this study is based on zoo tigers and lions. To assess the possible genetic variability in such animals from the natural habitats would entail collection of samples of their blood or bits of skin or even hair follicles. But for obtaining such samples from aggressive animals like tiger and lion, the animals have to be tranquilized. Wildlife experts avoid this because tranquilization puts the animal to considerable stress and strain. The body temperature of the animals shoots up and in order to keep the animals cool, water has to be constantly sprinkled over their bodies, otherwise there is the danger of their brain getting damaged, which may lead to death of the tranquilized animals. This is the standard practice when electronic collars are fixed on isolated animals for monitoring their movement etc. Furthermore, it may not be possible to practice such an invasive technique on a large scale for purposes of assessing genetic variability of a population in the wild.

On grounds of cruelty to animals, most wildlifers and animal lovers are opposed to collection of blood, skin and hair follicles involving tranquilization of the animals. Basically, these are invasive methods. Ranjit Talwar (WWF-India) is of the opinion that some method needs to be evolved so as to use *scats* (excrement) of felines. In this connection, while I was in Washington, Alka Sehgal and S. Ved Brat (Braton Brat) brought to my notice a paper on the use of coprocytobiology by Iyengar *et al.*[3] . The rationale behind using faecal matter is that millions of living epithelial cells are shed into faecal stream in stools of human being which can be recovered in viable state. Furthermore, such exfoliated cells from the walls of colon are present in the excreta of all animals, both carnivores and herbivores. These cells not only provide immediate past history of the colonic epithelium, but also have potential applications for biochemical studies, cancer research, mucosal immunity (immunology), medicine (gastroenterology), molecular and cellular biology, nutrition, and somatic cell genetics. The colonic cells after isolation have been used for mapping cell surface markers by flow cytometry; isolation of m-RNA, RT-PCR and studies on gene expression (insulin receptor has been isolated and cloned); isolation and characterization of cell surface glycoproteins; and isolation of DNA for studies on oncogenes, tumour-suppressor genes and for identifying mutations at specific gene loci[4]. Furthermore, since the original report published[3] in 1991, the procedure for isolation of colonic cells has undergone substantial modifications. The current methodology is both simple and far more rapid and cell yields in the range of 5–10 million per gram of stool have been obtained. DNA can be extracted for fingerprinting from these cells[4,5].

While methods for extraction of epithelial cells have been standardized in the case of human faecal matter, the same may have to be attempted in the case of carnivores and herbivores. Once such a technique is standardized, it will be possible to extract DNA from epithelial cells and work out DNA fingerprints. The technique has to avoid contamination on account of undigested cells from the prey species in the case of felines, and removal of large amount of fibre in the case of herbivores like elephants. DNA fingerprint profiles of such animals in the wild can be attempted at the population level. This would open the possibility to bring in genetic parameters in the realm of conservation of these mega wild animals, and base conservation on genetic evolutionary considerations. As indicated earlier, so far conservation of these animals has been based on increase in their numbers. Once this technique is adapted to study animals, it would provide a non-invasive method which does not involve taking their blood, bits of skin or hair follicles.

At present the world community of conservationists is looking to India for saving a magnificent animal like the tiger. This has to be accomplished on a scientific basis. It has become all the more necessary because WWF-International is not able to influence USA, Japan, UK and other European countries and Saudi Arabia to stop importing tiger-based medicines, lotions and potions from China and other manufacturing centres in SE Asia. Furthermore, so far it has also not been possible for WWF-International to press China to develop alternatives to tiger bones and/or help them to develop tiger farms where they could kill their own farm-raised tigers and extract bones for medicinal purposes and prepare soups of their genetalia and other body parts.

Techniques have also to be perfected to use very small quantities of herbarium material (without mutilating the herbarium specimens) to extract DNA and fingerprint the same so as to assess the extent and nature of genetic variation in selected plant species. Such information will be useful to evolve a strategy for endangered plants of economic importance for purposes of restocking, reintroduction, domestication and above all for *in situ* conservation.

In turn, such knowledge will also enhance understanding of phytogeography and zoogeography and movement of plants and animals in historical or geological time horizons. Equally important would be to understand inter-relationship of species, and origin and evolution of related assemblages of plants and animals and genetic changes accompanying the transition from wild to cultivated/domesticated condition of plants and animals and such other genetic-evolutionary problems. It may also be pointed out that

diversity exists at three levels: genes, species and ecosystems. These form a continuum and can be integrated into a hierarchical zoom. In selected cases there is also a need to understand diversity in such a context using simple but effective techniques like AFLP (amplified fragment length polymorphism), RFLP, RAPD, etc.

Lastly, the purpose of writing about mega-wildlife in *Current Science*[1] is to bring problems underlying this group of animals to the attention of professional conservation biologists, geneticists and molecular biologists of the country. The reason is that solutions to these problems lie well beyond the traditional wildlife practice.

1. Khoshoo, T. N., *Curr. Sci.*, 1997, **73**, 830–842.
2. Shankaranarayanan, P. *et al.*, *Electrophoresis*, 1997, **18**, 1693.
3. Iyengar, Y. *et al.*, *FASEB J.*, 1991, **5**, 2856–2859.
4. Nair, P. P., 1997, pers. commun.
5. Albaug, G. P. *et al.*, *Int. J. Cancer*, 1992, **52**, 347–350.

ACKNOWLEDGEMENTS. My thanks are due to Drs P. P. Nair (Beltsville) and Alka Sehgal and S. Ved Brat (Washington) for help with literature on the subject.

T. N. Khoshoo is in Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi 110 003, India.

SCIENTIFIC CORRESPONDENCE

Photochemical formation of gold nanoparticles in aqueous Triton X-100 and its application in SERS spectroscopy

Synthesis of gold nanoparticles is a challenging field because these crystallites have many uses. It has been used for staining proteins[1] electrotransferred onto nitrocellulose and also as a medium for surface enhanced Raman scattering (SERS)[2]. Colloidal gold, labelled to various biological materials like lectins, antibodies, antigens, enzymes, or lipoproteins enable these systems to be observed by transmission or scanning electron microscope. It can also show varying chemical reactivity[3] which strongly depends on the particle size. Gold sols also find use in the analytical chemistry for the determination of various polluting substances[4]. The gold sols are characterized by an attractive colour which can vary from ruby red through purple to blue depending on the size.

Gold hydrosol, containing particles of uniform size and regular spherical shape, is usually prepared by reducing $HAuCl_4$ with a solution of citric acid[5] or trisodium citrate[6]. The method for preparing gold sol by Moeremans *et al.*[7] is relatively troublesome and requires boiling and refluxing. The method due to Yamaguchi *et al.*[1] uses formalin but needs several hours stirring under appropriate pH. For maturation, it needed an additional vigorous overnight stirring. Generation of gold sol from gold(III) complex using other reducing agents such as ascorbic acid and hydrazine was also possible[4] but proper conditions were required. Poly(ethylene glycol) and poly(vinyl alcohol) were also recently used[8] for gold sol preparation but it took a long time.

Light-induced reactions could afford an ideal situation for many reactions[9,10]. For nanocystallite formation also, photochemical reduction could be an ideal alternative[11]. It has important advantages in comparison to the chemical reduction. Being homogeneous, it is reproducible and can be applied at ambient temperature. Photoinitiated reduction of gold(III) complexes has recently been accomplished with acetone and 2-propanol[12,13]. Here, acetone served as the sensitizer to absorb the UV light to initiate radical formation via the acetone triplet and subsequent hydrogen atom abstraction from 2-propanol. This type of photoreaction of phenols[14] or alcohols[15] is known in synthetic organic chemistry but is rarely applied for gold cluster preparation. Methanol also can serve as reducing agent[16] for gold sol preparation in a photochemical way.

Prevention of coagulation of small metal particles to form larger particles is another important criterion for nanoparticle generation. Chemically prepared metal colloids are only stable in solution, as they are protected by solvent molecules and electric charges, preventing coagulation. Addition of macro molecules like poly(vinyl alcohol) and poly(vinyl pyrrolidone)[17], co-polymers of vinyl alcohol and *N*-vinyl pyrrolidone[18], cyclodextrins[19], colloidal silicic acid[20], diallyl dimethyl ammonium chloride[16], sodium polyphosphate[12], etc. also worked well for this purpose.

Here we report a photochemical method for gold sol preparation using Triton X-100 (TX-100) in aqueous medium in a simple and quick way. Figure 1 shows the surface plasmon absorbance band (523 nm) of the zerovalent gold prepared by photoirradiation of 2 ml TX-100 (10^{-2} M) containing $HAuCl_4$ (150 ppm final concentration) for 20 min. More concentrated solutions of gold(III) can be used to obtain higher yields of gold particles. The irradiation was done with a germicidal lamp (15 W; Sankyo Denki, Japan). The samples were taken in well-stoppered 1-cm quartz cuvettes and were kept at a distance of 3 cm from the light source. Deoxygenation of the solution followed by irradiation for 20 min did not cause any change in the absorbance at 523 mm. The sol is stable for more than a month. Without TX-100, gold(III)

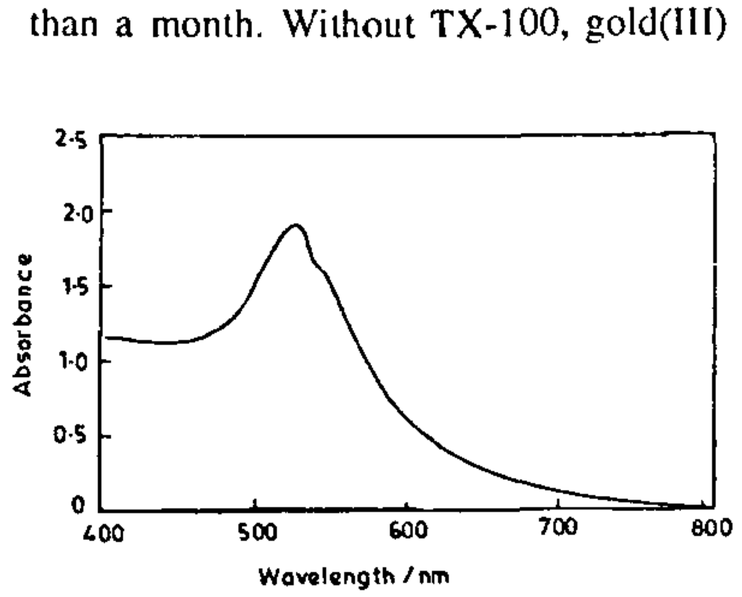

Figure 1. VIS spectra of gold hydrosol after 20 min irradiation.