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Abstract: In 1983, O'Brien *et al.* announced that cheetahs have remarkably little genetic variability. However, independent researchers, Caughley and Merola, studying 24 other carnivores, argued that cheetahs are not especially impoverished and deny that there is much evidence of any deleterious effects in the form of inbreeding depression. Current thinking may rightly recognize that lack of genetic diversity is not the primary factor for most endangered species. But O'Brien's concern nevertheless remains an important consideration for many conservation programmes, and particularly for cheetahs.

POPULATION GENETICS

The cheetah controversy

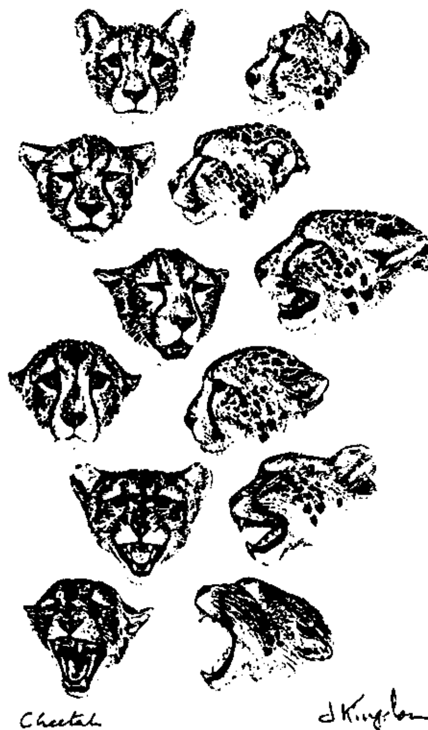
Robert M. May

IN 1983, O'Brien *et al.* created a stir with their paper announcing that cheetahs have remarkably little genetic variability[1,2]. At that time, much research centred on questions of minimum viable population sizes, and particularly on how small a population could become before lack of genetic variability would make its long-term survival unlikely[3]. These days, considerations of genetic diversity are rarely at the top of any list of factors currently causing species endangerment[4,5], so social historians will not find it surprising that O'Brien's work has been subjected to two reappraisals[5,6]. Both of the authors concerned, Caughley and Merola, argue that cheetahs are not especially impoverished genetically — and that it would be unlikely to matter much if they were.

The cheetah, *Acinonyx jubatus*, is found widely, if patchily, in sub-Saharan Africa, with a remnant population holding out in northern Iran[7]. The total cheetah population was estimated to be around 10,000 – 20,000 in the mid-1970s, having roughly halved over the preceding two decades as a result of hunting and changing land-management practices[8]. In Caughley's words, "how far they dropped further between 1975 and now is anyone's guess"[5].

In their early work, O'Brien and colleagues found no heterozygosity at any of 47 isozyme loci in 55 cheetahs from southern Africa. Subsequent studies extended the analysis to two-dimensional electrophoresis of some 155 soluble proteins, finding a mean heterozygosity of H = 0.014 for East African cheetahs (H measures the fraction of loci that are heterozygous in the average individual). A previously undetected isozyme polymorphism was also found in southern African cheetahs, giving $H = 0.0004$ for this population. This figure contrasts with an average heterozygosity of $H = 0.071 \pm 0.002$ for vertebrates, or (more relevant) $H = 0.067 \pm 0.005$ for a collection of 172 mammalian species[9]. O'Brien and collaborators have concluded that the cheetah passed through a genetic bottleneck some 10,000 years ago, and that a second bottleneck is responsible for the exceptional loss of genetic variability in southern African cheetahs (possibly caused by the arrival of humans in this region only relatively recently)[10].

Merola[6] brings together evidence for levels of genetic variation for 24 other species of terrestrial carnivores, and finds eight that show no heterozygosity (H = 0). The coyote, *Canis latrans*, has an H-value above the southern African cheetah (H essentially zero), but below the East African ones. The remaining 15 species all have H-values higher than cheetahs, with the overall average for this set of 26 carnivores being 0.028. Merola thus argues that the genetic variability of cheetahs is more or less typical of carnivores. She further emphasizes that carnivores generally have less genetic variability than most mammals (she compares them with a group of 81 other species, whose mean H value is 0.042; this contrast is even greater if we use H = 0.067 for a larger group of mammals[9], including carnivores). Caughley[5] independently makes broadly the same points, using much the same data.



Variability of cheetah facial expression, depicted by Jonathan Kingdon. The genetic variability of cheetahs is a more contentious (but less visual) matter.

In reply, O'Brien[11] emphasizes that Merola's and Caughley's comparison sets of H-values are based mainly on allozyme estimates of genetic diversity at a small number of loci. In particular, the assessments for the eight carnivore species rated by Merola as less genetically variable than cheetahs come from allozyme surveys of only 13 loci in the polar bear, *Ursus maritimus*, or 21 loci in the other seven species. The early cheetah studies of this kind used around 50 loci. But much electricity has flowed through gels since these early days, and O'Brien and coworkers' data are now based on more extensive studies, employing six additional measures: two-dimensional gel electrophoresis; acceptance or rejection of skin grafts among individuals (related to variability in the major histocompatibility complex, MHC); polymorphisms in length of restriction enzyme fragments within the MHC; similar studies of mitochondrial DNA; microsatellite polymorphisms; and fluctuating asymmetry in cranial measurements. Personally, I find the evidence from skin grafts and from fluctuating asymmetries a bit dodgy, but as a whole I think O'Brien's case is persuasive. The cheetah has markedly less genomic diversity than any other felid, including its closest relative, the puma (*Felix concolor*). The value of $H = 0.014$ is also low compared with other carnivore species studied in similar detail.

Even if the cheetah does indeed have an abnormally low degree of genetic variability, Merola and Caughley deny that there is much evidence of any deleterious effects in the form of inbreeding depression. O'Brien has argued that pronounced inbreeding depression shows up through high levels of abnormality in cheetah sperm, through litter sizes that seem comparatively small (averaging 1.5), through greater susceptibility to disease, and in general through the notable difficulty experienced by captive breeding programmes for cheetahs. Both Merola and Caughley contend that much of the difficulty in early captive breeding programmes was associated with differences between cheetah behaviour in the wild and in the constrained circumstances of zoos and preserves. Litter sizes in the wild range from 3 or 4 to as many as 5 or 6, and of 48 cub deaths reported in the wild, only one could have been attributed to genetic defects[6]. Nor are the sperm abnormalities off-scale for cats. Better management of programmes of captive breeding is leading to greater success.

Here I am inclined to side with the critics. Not least, there is other evidence that reduced genetic variability does not impair breeding success: today's healthy but relatively homozygous populations of Pere David's deer all spring from a very small founding population; and the bandicoot *Perameles gunnii* is widespread in Tasmania, despite its extreme homozygosity (interestingly, the relict population of fewer than 100 known individuals in mainland Australia shows quite high genetic variability[12]).

Both Merola and Caughley also argue that lack of genetic variability in the MHC may not put the cheetah at risk with respect to disease. O'Brien, however, points to an outbreak of feline infectious peritonitis virus in a wildlife park in Oregon; this mini-epizootic killed 19 cheetahs but none of the 10 similarly exposed lions. Merola dismisses this example on the grounds that the cheetahs were at much higher density than found in the wild. She goes further to speculate that infections of

this kind (microparasites, *sensu* May and Anderson[13]) may be less of a problem for carnivores, which, unlike most other mammals, tend to be solitary or occur as small groups at low densities; such diminished selection pressures could, she suggests, account for the systematically lower genetic diversity in the MHC in carnivores compared with other mammals. There is some sense in these speculations[13]. But they fail fully to appreciate O'Brien's main point, which is that relative homozygosity in the MHC — whether resulting from bottlenecks or, less likely, from selective considerations — can create problems with disease when such a population is brought into the relatively crowded conditions of captive breeding programmes, or when population densities increase in the wild. Cheetahs in Africa are indeed becoming more crowded: one cheetah per 6 km^2 in National Parks and other reserves, in comparison with a past figure of one per 100 km^2, according to one estimate[8]. So I think O'Brien's worries are well founded.

Merola's strongly expressed belief is that the cheetah's future is imperilled by loss of habitat and other consequences of human activities. She and Caughley see the lack of genomic variability as unimportant. O'Brien agrees — who could not? — that human effects are central to the cheetah's fate; he emphasizes, for instance, that cub mortality in the Serengeti is atypically high, partly because predator/prey ratios are higher there than in pristine settings, and partly because researchers working on cheetahs may inadvertently give other predators clues to the cubs' whereabouts[11].

In all, current thinking may rightly recognize that lack of genetic diversity is not the primary factor for most endangered species. But I share O'Brien's concern that it nevertheless remains an important consideration for many conservation programmes, and particularly for cheetahs. □

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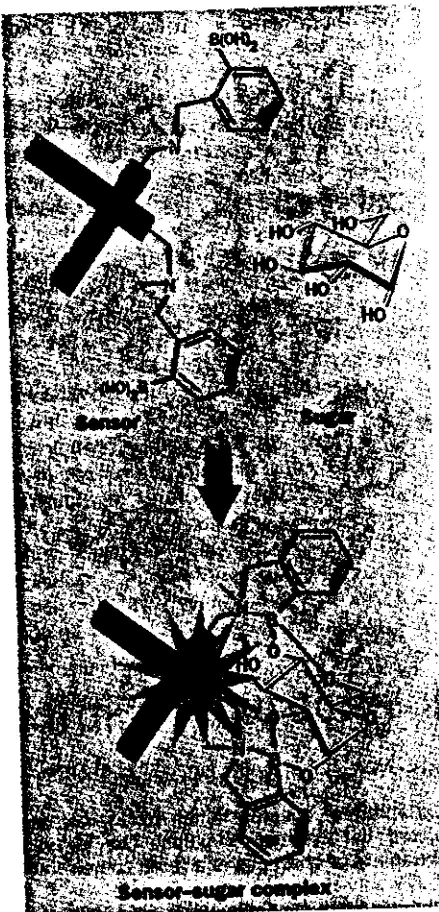
Bright spies for chiral molecul

A. Prasanna de Silva

LIKE people, molecules can be left- or right-handed, and this chirality is especially common among biologically important molecules. For instance, D-glucose is essential for energy production in an organism whereas L-glucose (its enantiomer or mirror image) would pass unused through its digestive system. A more tragic example is thalidomide, where one enantiomer acts as a drug for morning sickness whereas the other leads to serious deformities in the fetus. A practical method of selectively monitoring chiral molecules of this type would therefore be most welcome. On page 345 of this issue, James, Sandanayake and Shinkai[1] demonstrate that they have made good progress towards this goal by building a pair of fluorescent sensors that discriminate between sugar enantiomers.



A sensor of a given chirality binds preferentially with the sugar with the matching handedness and also produces a larger enhancement of fluorescence.

Optical monitoring of molecular chirality is not new. Indeed, a common manifestation of chirality is the rotation of the plane of polarized light by a solution of biologically active molecules. But this general approach, which includes sophisticated techniques such as o rotatory dispersion and circular d ism, lacks the high degree of sele required for the monitoring of a chiral molecule among many other complex matrix. This problem is esp ly serious for cases such as D-g because of its inability to absorb violet or visible light. One non- solution is to allow the natural e glucose oxidase to act on D-gluc the presence of air, and then dete resulting hydrogen peroxide e chemically[2]. Because it is so impor the control of diabetes, the monito D-glucose is now a profitable business.

A key to success in this and methods is the recognition capab the enzyme that selects D-glucos the myriad of other molecules pre whole blood. In the work of Jame molecular recognition between sug phenyl boronic acids has been give twist with the insertion of a chiral, cent 1,1'-binaphthyl as the back the sensor[1]. The inherent chiralit sensor lets it distinguish one suga tiomer from the other. Selectivity sugar over other sugars is achi building two terminal boronic ac the sensor for a pincer-grip.

The final part of the story is molecular event of selective recog D-glucose is translated into a comprehensible message. Fluore easily seen by the naked eye, can detected from a single molecul chemically switchable between 'off' states[3]. Fluorescent sensors ing by photoinduced electron (PET)[4] exploit these features monitoring of many chemical including sugars[5,6]. In many of t sors, the fluorescence increases upon binding with the chosen a

The present work of James longs to this family but is rather that it introduces chirality into fl PET sensing strategies for the

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