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.
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. 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. These days, considerations of genetic diversity are rarely at the top of any list of factors currently causing species endangerment, so social historians will not find it surprising that O'Brien's work has had a diminishing impact on our understanding of cheetahs.

Two authors of the book concerned, Caughley and Merola, argue that cheetahs are not especially impoverished genetically, and that this should 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. 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. In Caughley's words, "how far they dropped further between 1975 and now is anyone's guess.

In their early work, O'Brien and colleagues found no heterozygosity at any of 47 isozyme loci in 53 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 (it 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 $H = 0.004$ for this population. This figure contrasts with an average heterozygosity of $H = 0.071$ ± $0.022$ for 7 species of African carnivores (or more relevant) $H = 0.067 ± 0.005$ for a collection of 172 mammalian species O'Brien and colleagues 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 this species. The hypothesis of a bottleneck is strongly supported by the absence of heterozygosity at 45 of 53 loci in these cheetahs.

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 emphasizes that Merola and Caughley's comparison set $H$ 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 were based on 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 colleagues' data are 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 genome diversity than any other felid, including its closest relative, the puma (Felis 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 showing a comparitively 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 cubs deaths reported in the wild, only one could have been attributed to genetic defects. Nor are the sperm abnormalities over 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 homogenous populations of Peach 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 relic population of fewer than 100 known individuals in mainland Australia shows quite high genetic variability) 4. 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 Anderson1) 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 speculations2. 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 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 km² in National Parks and other reserves, in comparison with a past figure of one per 100 km², according to one estimate3. So I think O'Brien's worries are well founded.

Moler's strongly expressed belief is that the cheetah's future is imperiled by loss of habitat and other consequences of human activities. She and Caughley see the lack of genetic variability as unimportant. O'Brien agrees — who could not? — that human effects are central to the cheetah's fate; he emphasizes, for instance, that cubs mortality in the Serengeti is atypically high, partly because predators' 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' whereabouts4.

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.

Robert M. May is in the Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK.

14 A sensor of a given chirality binds preferentially with the sugar with the matching handedness and also produces a larger enhancement of fluorescence.
15 USED 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 CHERIAL MOLECULES OF THIS TYPE WOULD THEREFORE BE MOST WELCOME. ON PAGE 345 OF THIS ISSUE, JAMES, SADANAYAKE AND SHINKLA DEMONSTRATE THAT THEY HAVE MADE GOOD PROGRESS TOWARDS THIS GOAL BY BUILDING A PAIR OF FLUORESCENT SENSORS THAT DISCRIMINATE BETWEEN SUGAR ENANTIOMERS.
16 OPTICAL MONITORING OF MAMMALIAN 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 more sophisticated techniques such as optical rotatory dispersion and circular dichroism, lacks the high degree of sensitivity required for the monitoring of a specific chiral molecule among many others in a complex matrix. This problem is especially serious for cases such as D-glucose because of its inability to absorb ultraviolet or visible light. One non-optical solution is to allow the natural enzyme D-glucose oxidase to act on D-glucose, resulting in the presence of air, and then detect the resulting hydrogen peroxide electrolytically.
17 Because it is so important to the control of diabetes, the monitoring of D-glucose is now a profitable medical business.
18 A key to success in this and related methods is the recognition capability of the enzyme that selects D-glucose from the myriad of other molecules present in the whole blood. In the work of James et al., the molecular recognition between sugars and their related boronic acids has been achieved with a twist in the insertion of a chiral, fluorophore 1,1'-binaphthyl as the backbone of the sensor. The inherent chirality of this sensor lets it distinguish one sugar enantiomer from the other. Selectivity for the sugar over other sugars is achieved by building two terminal boronic acids into the sensor for a pincer-grip.
19 The final part of the story is how the unique molecule of selective recognition D-glucose is translated into a human comprehensible message. Fluorescence is easily seen by the naked eye, can even be detected from a single molecule an chemically switchable between 'on' and 'off' states. Fluorescent sensors opening by photoinducded electron transfer (PET) exploit these features for monitoring of many chemical species including sugars. In many of these sensors, the fluorescence increases on binding with the chosen analyte.
20 The present work of James et al. long to this family but is rather specific that it introduces chirality into fluorescent PET sensing strategies for the first time.