Giant Panda Paternity

We have determined by biochemical genetic procedures that the baby giant panda (*Ailuropoda melanoleuca*) born at the National Zoological Park in July 1983 was the offspring of the Washington male (Hsing-Hsing) and have excluded the possible parentage by the London male, Chia-Chia. The question arose because the mother, Ling-Ling, successfully mated with Hsing-Hsing (for the first time!) in March 1983 and was artificially inseminated with sperm from Chia-Chia on the 2 days following the sexual encounter.

Ling-Ling first showed signs of sexual receptivity in the spring of 1973 and has demonstrated estrus once a year ever since. Although Hsing-Hsing is a rather healthy male, his sexual encounters with Ling-Ling through the 1970's were unsuccessful due to what has been politely termed an orientation problem (*1*).

In 1980, artificial insemination was attempted on 2 days of estrus using fresh spermatozoa collected from Hsing-Hsing by electroejaculation (*2*), but no pregnancy resulted. In 1981, the London male giant panda, Chia-Chia, was flown to Washington for a mating encounter with Ling-Ling. This pairing resulted in an aggressive and extended physical attack on Ling-Ling by Chia-Chia, which did not include copulation. In 1982, Ling-Ling was again artificially inseminated on each of 3 days of estrus. Frozen-thawed spermatozoa from Chia-Chia were used on the first day, and fresh spermatozoa from Hsing-Hsing were used on the second and third days. During this estrus Ling-Ling was subjected to two laparoscopic examinations at 48-hour intervals so that ovarian activity could be evaluated. Although the reproductive organs appeared morphologically normal and follicular luteinization was detected, the artificial insemination was not successful in producing a panda cub.

On 17 March 1983, Ling-Ling displayed symptoms of estrus again; on 18 March, Hsing-Hsing successfully mounted and copulated. On 19 and 20 March, fresh buffered spermatozoa was collected at the London Zoo from Chia-Chia and the specimens were used for artificial insemination. On 21 July, Ling-Ling gave birth to a male cub weighing 134 grams. Approximately 3 hours after birth, the cub died. Eleven hours later, the cub was removed from the cage, an autopsy was performed, and the cause of death was determined to be acute pneumonia that had developed prenatally from an ascending *Pseudomonas* infection in the birth canal.

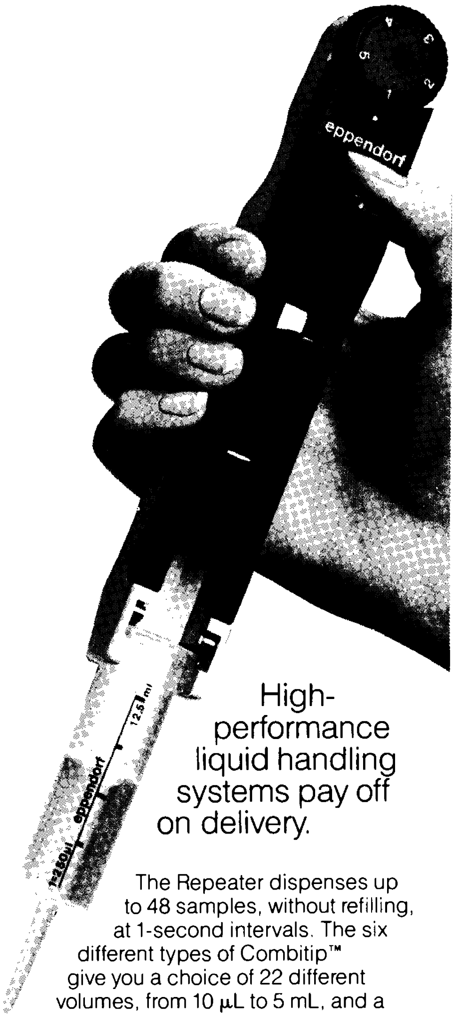
In order to establish paternity for the baby panda, a blood sample and a skin biopsy were obtained on 21 July and the skin was placed in tissue culture at the National Institutes of Health. Blood and skin biopsies from Ling-Ling, Hsing-Hsing, and Chia-Chia were also collected and placed in cell culture. As there is nothing known of biochemical genetic variation in this species, we used two techniques to detect informative polymorphic gene products. The first was to sample 29 isozyme loci, 22 of which we have designated as mammalian "polymorphic cluster" enzyme loci because they tend to be polymorphic in other mammalian species (*3*). They were ACP1, ACP2, ADA, ESA, ESD, FUCA, GALA, GALB, GOT1, GOT2, G6PD, GPI, GSR, GLO, HK1, HK2, IDH1, MDH1, ME1, PEPA, PEPB, PEPC, PEPD, PGD, PGM1, PGM3, PP, SOD1, and MPI (*4*). Surprisingly, none were polymorphic in our group of four giant pandas.

The second approach consisted of the separation at high resolution of abundant soluble proteins in two dimensions (2DE) on polyacrylamide gels (*5*). Proteins from the four cell lines (from baby, Ling-Ling, and the two suspect fathers) were labeled with ^{14}C amino acids and subjected to 2DE gels and autoradiography plus silver staining for detection of proteins. The autoradiographic patterns were analyzed by computer-assisted densitometry (*6*). Of the approximately 300 proteins examined, six exhibited quantitative polymorphism within the sample of four. Each polymorphism involved a mobility shift due to charge alteration, and all the putative polymorphisms exhibited gene dosage dependence in heterozygotes which is consistent with a genetic basis (*5*). The phenotypes of the four animals at each of the six loci, designated p90, p68, and so forth, after their molecular weights ($\times 10^{-3}$) appear in Table 1. Two of the loci, p90 and p73, show that the only way that the baby could have been Ling-

Table 1. Panda phenotypes. Abbreviations: A, acidic allele homozygote; B, basic allele homozygote; and AB, heterozygote.

Protein	Ling-Ling	Baby	Hsing-Hsing	Chia-Chia
p90*	A	AB	AB	A
p68	AB	B	B	B
p32	AB	AB	B	B
p73*	B	AB	AB	B
p112	AB	AB	A	AB
p54	A	A	A	AB

*Informative loci.



Ling's son is if Hsing-Hsing had been the father. Chia-Chia is excluded by both loci. The genotypes of the four other loci are also consistent with this explanation.

These results are reassuring insofar as they confirm the virility and fertility of Hsing-Hsing and add another breeding male to the very small population of captive male giant pandas.

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3. S. J. O'Brien, M. Gail, D. L. Levin. *Nature (London)* **288**, 580 (1980).
4. Abbreviations are as follows: ACP1, erythrocyte acid phosphatase; ACP2, tissue acid phosphatase; ADA, adenosine deaminase; ESA, esterase-A; ESD, esterase-D; FUCA, α-L-fucosidase; GALA, galactosidase-A; GALB, galactose-B; GOT1, glutamate oxaloacetate transaminase-1; GOT2, glutamate oxaloacetate transaminase-2; G6PD, glucose-6-phosphate dehydrogenase; GPI, glucose phosphate isomerase; GSR, glutathione reductase; GLO, glyoxalase-1; HK1, hexokinase-1; HK2, hexokinase-2; IDH1, isocitrate dehydrogenase-1 (soluble); MDH1, malate dehydrogenase-1 (soluble); ME1, malic enzyme 1 (soluble); PEPA, peptidase-A; PEPB, peptidase-B; PEPC, peptidase-C; PEPD, peptidase-D; PGD, 6-phosphogluconate dehydrogenase; PGM1, phosphoglucomutase-1; PGM3, phosphoglucomutase-3; PP, pyrophosphatase, inorganic; SOD1, superoxide dismutase-1; and MPI, mannose phosphate isomerase.
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7. Copies of gel photographs are available by request from the authors. The excellent technical assistance of Mary Eichelberger and Janice Martenson is gratefully acknowledged.

Ediacaran Fossils

In his article "Alien beings here on Earth" (Research News, 6 Jan., p. 39), Roger Lewin reports on the views of Adolf Seilacher of Tübingen University with respect to fossils occurring in the Late Precambrian Ediacaran beds of South Australia. Seilacher is said to believe that these fossils have a completely different architecture and physiology from Phanerozoic (Cambrian-Recent) organisms. It is reported to be his view that these body fossil organisms of some 670 million years ago died without descendants.

Many of the Ediacaran body fossils have already been described and attributed without exception to extant phyla of marine organisms but, according to Seilacher, one can question these attributions for two reasons. He says that the Ediacaran sediments are coarser than those which, in the Phanerozoic, contain preserved evidence of the groups involved. This is especially so in the case of soft-bodied organisms, as most of the described Ediacaran fauna are. He thinks the Proterozoic organisms were invested with a tougher integument than later organisms (and hence were better preserved in coarse sediments) and had primordial flattish and "quilted" surface, elsewhere unknown. Because he found no evidence of a mouth in some of the organisms heretofore attributed to Vermes, Seilacher concludes they were mouthless (and presumably gutless) and that their seeming metamerism is an illusion; he speculates that they were possibly acellular and carried on their physiologic processes directly by transfusion through their thick integumental wall. It should be pointed out, however, that it is unusual indeed to find the mouth area preserved in rare fossil worms which, by their segmented nature, invite disassociation after death; and pray how, other than flattened, would one expect to find a worm preserved? Or any other essentially soft-bodied creature?

Mine is the traditional view that the Ediacaran fauna is a backward projection of the Cambrian shallow benthos requiring no exceptional explanations of any sort. In the more than 130 million years between the Ediacaran time and the earliest Cambrian, hard parts (calcium phosphate, calcium carbonate, chitin), which may have been incipient in the Ediacaran organisms, were widely developed by invertebrate creatures. This made their basal Phanerozoic preservation not only abundant, but so much so by way of contrast as to seem to some paleontologists to have been the result of essentially instantaneous "explosive" evolution. In contrast to Seilacher's attribution of the known Ediacaran fauna to a "world apart," most of it seems to me to fit comfortably into extant phyla.

In the earliest Cambrian, many of the organisms seem to have been at that time "living fossils" which necessarily had had a long antecedent, but undiscovered, evolutionary history. Current work on primitive arthropods (trilobites and non-trilobites) furnishes considerable evidence of this nature. The presence also in the Early Cambrian of the remains of several higher arthropod lineages, some