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A Complex Chromosome Rearrangement in the Karyotype of a Wild-Caught Male Sea Otter

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California sea otters have been maintained at Sea World, San Diego, California since 1972. The sea otters studied here had been integrated into a sea otter breeding program in 1973 (Antrim and Cornell 1980). The early pupping history of this breeding colony, when it consisted of the original male and eight females, was characterized by a pattern of stillbirths, anomalous offspring and spontaneous abortions. The congenital anomalies expressed included a heart defect, the absence of the digestive tract from mouth to intestines with other instances of parts of the digestive tract missing (e.g., liver, pancreas), incomplete diaphragm and the presence of facial anomalies, such as severe cleft palate and absence of eyes. The severity and array of the abnormalities were, to us, highly suggestive of the types of birth malformations seen in the segregation of chromosome anomalies in humans (Yunis 1977; Smith and Jones 1982). Chromosome studies were, therefore, undertaken on the male and three of the females in the breeding colony to investigate the possibility that there was a chromosomal abnormality segregating in this breeding colony. We report here the results of chromosome studies undertaken on a male and 3 female California sea otters in the Sea World sea otter breeding colony. We also discuss possible implications of these findings to conservation efforts.

Materials and methods

Karyotypes were established from blood and fibroblast cultures in the male, and from blood cultures in the females. At the time of this study, no pups were available for examination. Blood was drawn by femoral venipuncture. Tissue and blood samples were refrigerated and shipped by one-day air to Portland State University for culturing. Blood samples were cultured in RPMI-1640 media (Gibco), supplemented with 25% fetal calf serum, antibiotics and phytohemagglutinin (Duffield and Chamberlin-Lea 1990). These cultures were grow for 4 days, synchronized for one to two hours with colcemid, followed by a second 6-10 minute colcemid synchronization 21 hours later. The cells were harvested, exposed to 0.075M KCL hypotonic, and fixed. Subsequent to the initial blood sample, the male died of septic endotoxemia and corneal fibroblasts were cultured. The fibroblast culture was grown in Hams F-10 (Gibco), supplemented with 15% fetal calf serum, antibiotics and a fungicide, as per Duffield et al. (1991).

A variety of staining techniques were used to construct karyotypes. These included standard Giemsa staining, G-banding, C-banding (Hack and Lawce 1980), and a fluorescent R-banding technique using chromomycin A3 and distamycin A (Sahar and Latt 1978; Schweizer 1980; Duffield and Chamberlin-Lea 1990; Duffield et al. 1991).

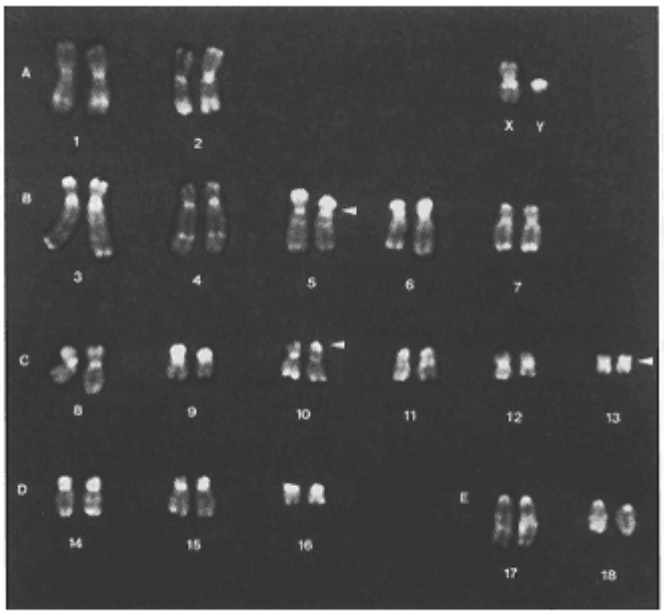

Figure 1. An R-banded karyotype of a female sea otter (2N = 38). Chromosomes are grouped A through D and numbered based on size and centromere position. The X chromosomes are indicated.

Results

Chromosome irregularities were seen in the male California sea otter karyotype when compared with the three females. The chromosomal differences in the male were consistently present in all of his cells, both leucocyte and fibroblast, and were not seen in any of the cells of the females examined. A representative female R-banded karyotype is shown in Figure 1; the male is presented in Figure 2.

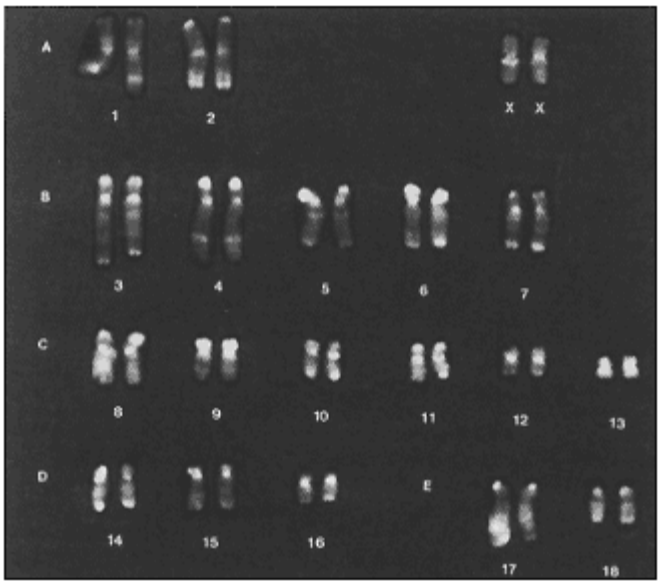

Figure 2. An R-banded karyotype of the male sea otter in question in this study (2N = 38). The chromosome pairs exhibiting consistent irregularity are indicated by arrows.

The three pairs of chromosomes exhibiting a consistent irregularity in the male (b5, c10 and c13) are marked. The differences between the chromosome pairs in the male and in the three females is illustrated in more detail in Figure 3.

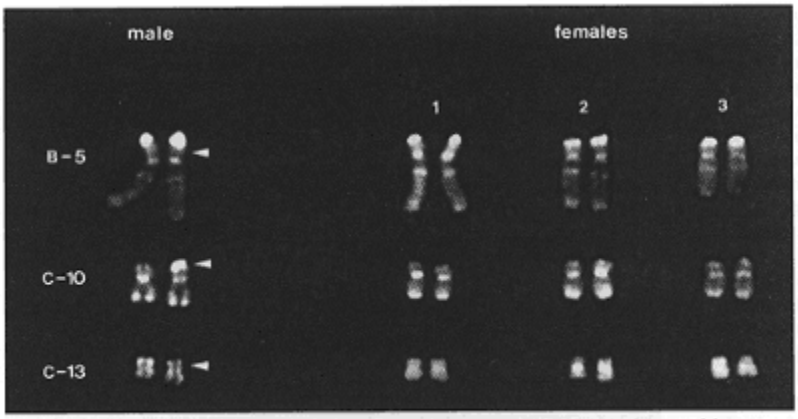

Figure 3. The abnormal homologues in the breeding male compared with the homologues of those same chromosome pairs in the three females examined in this study. The areas indicate regions of irregularity.

The rearranged b5 homologue in the male is missing a faint-staining or 'dull' area in the short arm. The c10 rearrangment represents a possible inversion of the short arm of the chromosome (see Figure 4) and there is an apparent difference in the short arm and centromere region of one of the chromosomes of pair c13, as compared to the other homologue and to the chromosomes of the females.

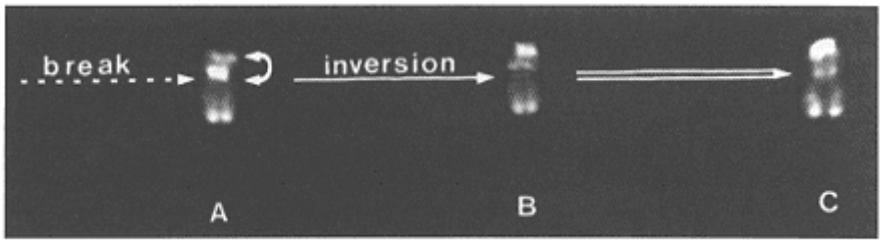

Figure 4. A proposed inversion explaining the rearrangement in c10 in the male sea otter. The inversion was modeled by inverting the equivalent part of the short arm of one of the c10 chromosomes in a female karyotype (A - B) and comparing it visually to the "rearranged" chromosome in the male (C).

Discussion

The precise nature of the chromosomal changes in the male California sea otter are not easy to resolve without chromosomal evaluation of the abnormal offspring. Many species of marine mammals have variable fluorescent R-band regions, referred to as heteromorphisms (Duffield and Chamberlin-Lea 1990; Duffield et al. 1991). These variable regions can differ between individuals within populations and are considered to be normal variants in the chromosomes . The apparent inversion in chromosome c10 could, indeed, be an example of normal R-band differences in the centromeric and short arm telomeric region of this chromosome. However, this variant was not seen in any of the females, nor in another male karyotyped subsequent to this study (this male was chromosomally similar to the three females and did not exhibit any karyotypic irregularities). It was judged to be an inversion because of the way it visually matched an "inversion" produced by physically inverting the short arm of chromosome c10 in the females (see Figure 4). However, only a small number of California sea otters have been examined and future karyotyping of more animals would help to resolve the normal vs. abnormal interpretation of this particular chromosome. The types of changes in the other two chromosome pairs have not been seen as normal chromosome variants in other marine mammal species studied with R-banding, and from the anomalies seen in the pups sired by this male, it would seem likely that there was, indeed, a complex chromosomal rearrangement in the karyotype of the breeding male. Since this wild-caught male was phenotypically normal, it was presumed that he was a balanced carrier for this rearrangement. Balanced complex de novo chromosome rearrangements have been reported in humans (e.g., Magenis et al. 1981).

The pupping history in the sea otter colony for the period involved the breeding of this male and the eight females. Eighteen pups, all sired by the one male present in the breeding colony, were born from 1975 to 1982. There was one set of twins. Eleven pups were stillbirths or abortuses. Several of these exhibited congenital anomalies. The anomalies were not confined to particular matings, but appeared in offspring of several of the females. Of the live births, five pups lived only 1-2 days. One was congenitally abnormal, the others were either under normal birth weight (as compared to values of 1.4 - 2.3 kg reported for successful births in wild sea otters: Kenyon 1969; Estes 1980) and congenitally weak, or appeared malnourished and died of maternal neglect. There were three pups of good birth weight, who lived for a longer period of time. One female pup lived for approximately two weeks and died of a respiratory infection. Another female pup died after 21 days of malnutrition and maternal neglect. One male pup lived for four months, dying of congestive heart failure resulting from an apparent congenital heart defect. Interbirth intervals ranged from 7 months 25 days to over 31 months, with a median interbirth interval of 11 months. Therefore, in this breeding colony where there was continual access to a male, there is the possibility of additional undetected pregnancies and spontaneous abortions during the apparently longer interbirth intervals.

Given the normal chromosome pairs seen in the karyotypes of the females examined, the existence of the chromosomal irregularities in the male karyotype suggested that he was a major contributor to the reproductive problems in the breeding colony. Numerous examples of chromosomal rearrangements producing multiple congenital anomalies are described in humans (Yunis 1977; Smith and Jones 1982). With the increased chromosomal resolution made possible by chromosome banding techniques, many cases of fetal and newborn abnormalities and malformations, and of increased rate of spontaneous

abortion and stillbirth, have been associated with families carrying balanced chromosome rearrangements, including both simple and complex translocations and inversions (Budberg et al. 1982; Buchinger et al. 1981; Anderson et al. 1981; Cohen et al. 1983; Ostovics et al. 1982; Palmer et al. 1987; Gadow et al. 1991). The occurrence of late pregnancy abortions, failure to thrive, maternal neglect and the range of abnormalities seen in the offspring in this breeding colony would be consistent with a chromosome rearrangement involving small chromosome segments.

Interpretation of the observed congenital anomalies as directly resulting from genetic missegration of a complex chromosome rearrangement in the male is complicated by two factors. At the time of this study, the offspring could not be karyotypically examined to associate specific chromosomal duplication or deficiencies with the presence of anomalies. Furthermore, there is evidence both from captive breeding of sea otters (Antrim and Cornell 1980) and from the wild (Kenyon 1969), that first-time births have a lower success rate and are often characterized by stillbirths or failure of the pups to thrive. These effects would have also contributed to the pupping history in this breeding colony. To this latter point, however, the consistent pattern of congenital anomalies, stillbirths and spontaneous abortions throughout the breeding history of a number of the females, argues against this being the only explanation for the breeding problems. The most common signs of autosomal chromosome aberrations, which include increased spontaneous and stillbirth rates, and in live offspring, low birth weight, failure to thrive, mental retardation, head and facial anomalies and heart and abdominal defects (Vogel and Motulsky 1979; Gadow et al. 1991), certainly characterize the pupping history in this colony.

Implications for conservation efforts

Congenital malformations can also be caused by a number of environmental factors. The possibility that the birth anomalies were caused by a teratogenic agent cannot totally be ruled out in the absence of verification of karyotypic abnormalities in the anomalous offspring. However, it can be argued that given the range of congenital defects, as well as the presence of apparently phenotypically normal offspring in this colony, one would not expect the problem to be related to a single teratogenic agent, nor to any single, consistent factor in their environment. A study of the water environment of this sea otter colony was undertaken, subsequently to the chromosome study, to see whether the water quality treatment or filtering of water in this particular system produced a teratogenic-like effect on in vitro cells (Nicholas and Duffield, unpublished observation). Cells were grown in water from a variety of sources and were scored for chromosome damage; such as, breaks, rearrangements and sister chromatid exchanges. The highly treated water of the sea otter filtration system, compared with other sources, which included purified water, local bay water and local drinking water, was ranked as having no added effect on chromosomal change in culture when compared to the purified water control. It is interesting, as an aside, that the local drinking water had a statistically significant effect on chromosome breakage and rearrangement in the cultures.

Potentially, there is also the possibility that the apparent "irregularities" in the chromosome pairs of the male resulted from a cross between parents from stocks of wild sea otters which were chromosomally differentiated. For example, there has been continuing controversy over the discreteness of Alaskan and Californian sea otter populations (Kenyon 1969; Lidicker and McCollum 1981; Wilson et al. 1991; Cronin et al. 1996). The male in question came from Monterey Bay, California. However, with the occasional introduction of Alaskan sea otters along the Oregon and Washington coasts (see Jameson et al. 1982), there is a possibility, although thought to be remote, that some of these have been integrated into the California population. If these two populations were chromosomally distinct, a cross between them would certainly give rise to an individual exhibiting irregular pairing of chromosomes. An R-band karyotype has not yet been done for the Alaskan sea otter to test this possibility. Either source, structural rearrangement or population difference, could have resulted in the production of anomalous offspring by the carrier animal.

Ralls et al. (1983) predicted that despite the population bottleneck in the California sea otter population caused by fur hunting in the 18th and 19th centuries, 77% of the original genetic diversity would be

expected to be retained and that the bottleneck would, therefore, not have had much impact on loss of genetic variation. Genetic variation in both allozymes and mtDNA has been subsequently demonstrated in various sea otter populations (see Cronin et al. 1996; Scribner et al., in press). This would imply that deleterious effects, such as, increased mortality in young animals and reduced fertility expected as a result of loss of genetic variability, would not be an anticipated consequence of the near extinction of the California population (Ralls et al. 1983; Cronin et al. 1996). On the other hand, from a genetic drift argument, if one of the subsequent founders of the current population, i.e. one of the survivors, was carrying a chromosome rearrangement, the frequency of that rearrangement in the recovered population could be fairly high based on chance contributions of individual founders. In time, selection would be expected to lower the frequency of the chromosome rearrangement, but in the interim, because of its effect on reduced fertility (Tupler et al. 1994) as well as on lowered pup production through spontaneous abortion and failure to thrive, it might have a significant impact on the observed rate of recovery of the population. This would be true, as well, if the source of chromosome irregularity were the consequence of breeding between individuals of chromosomally differentiated subspecies subsequent to sea otter translocation efforts.

The frequency with which balanced chromosome rearrangements occur in the wild is not known for most species. In man, the frequency of balanced autosomal rearrangements estimated from prenatal cytogenetic studies ranges from 0.224% (Hook et al. 1983) to 0.4% (Van Dyke et al. 1983). Chromosome abnormalities are reported for cattle (Swartz and Vogt 1983; Bongso and Basrur 1976), sheep (Bruere 1969), pigs (Akesson and Henricson 1972; Henricson and Backstrom 1964) and horses (Hughes and Trommershausen-Smith 1977). These have been associated with physical anomalies, fetal death and reproductive inefficiency. Balanced translocations have been reported for wild-caught cetaceans (Duffield 1977; Worthen 1981). The presence of a carrier of a chromosomal rearrangement in a wild sea otter is, therefore, not in itself, surprising. However, in a population which has suffered the degree of genetic bottlenecking and reduction in numbers seen with the California sea otter, it might be useful to further evaluate the frequency of chromosomal rearrangements in the current population.

In terms of the impact on captive breeding programs, the finding of a phenotypically normal, but karyotypically abnormal wild-caught male in the Sea World breeding colony, emphasizes the importance of screening potential breeding animals for chromosomal rearrangements which could lead to costly risk of abnormal births and compromised breeding success in captive breeding programs. This is especially important in breeding colonies built around a single male or a single breeding pair of animals.

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Literature Cited

Akesson, A. and B. Henricson. 1972. Embryonic death in pigs caused by unbalanced karyotypes. Acta Vet Scand. 13:151-160.

Anderson, O., C. Lundsteen, and E. Niebukri. 1981. A complex four break rearrangement between chromosomes 4 and 13 in a recombinant chromosome 4. Cytog. Cell Genetics 30:3-10.

Antrim, J.E. and L.H. Cornell. 1980. Reproduction of the sea otter. International Zoo Yearbook 20:76-80.

Bongso, A. and P.K. Basrur. 1976. Chromosome anomalies in Canadian Guernsey bulls. Cornell Vet.

66:476-489.

Bruere, A.N. 1969. Male sterility and an autosomal translocation in Romney sheep. Cytog. 8:209-218.

Buchinger, G., A. Wettstein, and H. Metze. 1981. Familial chromosome translocation t(3:18)(p21;p11). Journal of Med. Genetics 18:119-123.

Budberg, A., L. Iselius, L. Lindgren, and K.M. Lundmark. 1982. Duplication of part of the long arm of chromosome 8 due to a familial 8:13 translocation. Hereditas 96:105-108.

Cohen, M.M., J. Charrow, N. Balken, and C. Harris. 1983. Partial trisomy 14 (q23-qter) via segregation of a 14/X translocation. American Journal of Human Genetics. 35(4):635-645.

Cronin, M.A., J. Bodkin, B. Ballachey, J. Estes, and J.C. Patton. 1996. Mitochondrial-DNA variation among subspecies and populations of sea otters (Enhydra lutris). Journal of Mammalogy 77 (2):546-557.

Duffield, D.A. 1977. Phylokaryotypuc evaluation of the cetacea. Ph.D. dissertation. University of California, Los Angeles, California. 154 pp.

Duffield, D.A. and J. Chamberlin-Lea. 1990. Use of chromosome heteromorphisms and hemoglobins in studies of bottlenose dolphin populations and paternities. Pages 609-622 in S. Leatherwood and R.R. Reeves, editors. The Bottlenose Dolphin. Academic Press, San Diego, California.

Duffield, D.A., J. Chamberlin-Lea, J.C. Sweeney, D.K. Odell, E.D. Asper, and W.G. Gilmartin. 1991. Use of corneal cell culture for R-band chromosome studies on stranded cetaceans. Pages 91-100 in J.E. Reynolds and D.K. Odell, editors. NOAA Technical Report NMFS 98.

Estes, J.A. 1980. Enhydra lutris. In: Mammalian Species. American Society of Mammalogy. No. 133:1-8.

Gadow, E.C., S. Lippold, L. Otano, E. Serafin, R. Scarpati, and T. Matayoshi. 1991. Chromosome rearrangements among couples with pregnancy losses and other adverse reproductive outcomes. American Journal of Med. Genetics 41:279-281.

Hack, M.S. and H.J. Lawce, editors. 1980. The association of cytogenetic technologists cytogenetics laboratory manual. Association Cytog. Techs. Inc., 677-S, University of California, San Francisco, California. 430 pp.

Henricson, B. and L. Backstrom. 1964. Translocation heterozygosity in a boar. Hereditas 52:166-170.

Hook, E., Schrimmacher, Willey, and Cross. 1983. Rates of mutant structural chromosomal rearrangements in human fetuses; data from prenatal cytogenetic studies and associations with maternal age and parental mutagen exposure. American Journal of Human Genetics 35:96-109.

Hughes, J.P. and A. Trommershausen-Smith. 1977. Infertility in the horse associated with chromosomal abnormalities. Aust. Vet J. 53:253-257.

Jameson, R., K.W. Kenyon, A.M. Johnson, and H.M. Wright. 1982. The history and status of translocated sea otter populations in North American Wildlife Society Bulletin 10:100-107.

Kenyon, K.W. 1969. The sea otter in the eastern Pacific ocean. North American Fauna, Number 68. 352

pp.

Lidicker, W.Z. and F.C. McCollum. 1981. Genetic variation in the sea otter, Enhydra lutris. Fourth Biennial Conference on Biology of Marine Mammals, San Francisco, California. (Abstract).

Magenis, E., M.G. Brown, J. Chamberlin, T. Donlon, D. Hepburn, N. Lamvik, E. Lovrien, and M. Yoshitomi. 1981. Resolution of breakpoints in a complex rearrangement by use of multiple staining techniques: confirmation of suspected 12p12.3 intraband by deletion dosage effect of LDH-B. American Journal of Human Genetics 9:95-103.

Ostovics, M.K., S.P. Toth, and J.A. Wessely. 1982. Cytogenetic investigations in 419 couples with recurrent fetal wastage. Ann. de Genetique 25 (4):232-236.

Palmer, C.G., J.H. Miles. P.N. Howard-Peebles, R.E. Magenis, S. Patil, and J.M. Friedman. 1987. Fetal karyotype following ascertainment of fetal anomalies by ultrasound. Prenatal Diagnosis 7:551-555.

Ralls, K., J. Ballou, and R.L. Brownell, Jr. 1983. Genetic diversity in California sea otters: theoretical considerations and management implications. Biological Conservation 25:209-232.

Sahar, E. and A. A. Latt. 1978. Enhancement of the banding patterns in human metaphase chromosomes by energy transfer. Proceedings of National Academy of Science U.S.A. 75 (11):5650-5654.

Schweizer, D. 1980. Simultaneous fluorescent staining of R-bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. Cytog. and Cell Genetics 27:190-193.

Scribner, K.T., J. Bodkin, B. Ballachey, S.R. Fain, M.A. Cronin, and M. Sanchez. In press. Population genetic studies of the sea otter (Enhydra lutris): a review and interpretation of available data. U.S. Department of Commerce, National Oceanic and Atmospheric Administration Technical Report.

Smith D.W. and K.L. Jones. 1982. Recognizable patterns of human malformation: genetics, embyrologic and clinical aspects. In M. Markowitz, editor. Series - Major problems in clinical pediatrics. Vol. VII. W.B. Saunders, Philadelphia, Pennsylvania.

Swartz, H.A. and D.W. Vogt. 1983. Chromosome abnormalities as a cause of reproductive inefficiency in heifers. Journal of Heredity 74:320-324.

Tupler, R., L. Barierato, D. Larizza, P. Sampaolo, F. Piovella, and P. Maraschio. 1994. Balanced autosomal translocations and ovarian dysgenesis. Human Genetics 94:171-176.

Van Dyke, D.L., L. Weiss, J.R. Roberson, and V.R. Babu. 1983. The frequency and mutation rate of balanced autosomal rearrangements in man estimated from prenatal genetic studies for advanced maternal age. American Journal of Human Genetics 35 (2):301-307.

Vogel, F. and A.G. Motulsky. 1979. Human genetics, problems and approaches. Springer-Verlag, New York, New York. 700 pp.

Wilson, D.E., M.A. Bogan, R.L. Brownell, Jr., A.M. Burdin, and M.K. Maminov. 1991. Geographic variation in sea otters, Enhydra lutris. Journal of Mammology 72 (1):22-36.

Worthen, G.L. 1981. Preliminary analysis of the potential for stock assessment of Pacific ocean Delphinids by G and C chromosome banding. National Marine Fisheries Service Administrative Report

LJ-81-02C. 99 pp.

Yunis, J.J. 1977. New chromosomal syndromes. Academy Press: New York, New York. 404 pp.

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