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MATERNAL TRANSMISSION OF MITOCHONDRIAL DNA IN DUCKS

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Maternal transmission of mitochondrial DNA (mtDNA) has been studied in amphibians, insects and mammals, but little is known about mtDNA inheritance in the ovaripirous avian species. In this study, we have constructed the physical maps of mitochondrial genomes from two different genera of ducks (Cairina and Anas) and taken advantage of the availability of their hybrids to demonstrate that mtDNA is maternally inherited.

Mitochondrial DNA (mtDNA) has been suggested to be maternally inherited in Xenopus (1) and Drosophila (2,3). Maternal passage of mtDNA has also been inferrred in squirrels (4), musk shrew (5), Deer mice (6), Rattus rats (7,8,9), and white footed mice (10) as well as horse-donkey hybrids (11). With restriction fragment length polymorphism, additional markers are now available to show that mtDNA is maternally transmitted in the human populations (12-14).

Except for an early study comparing the physical maps of chicken mtDNA and those of other related species (15), investigations on avian mt genomes have been confined to systematics and evolutionary relationships (16-18). This report shall show by restriction enzyme mapping and comparison that there is a distinct difference between the mtDNA of two different genera of ducks and that their mtDNA inheritance is strictly maternally determined.

MATERIAL AND METHODS

Isolation of mtDNA

Male Mascovy (Cairina muschata), female Tsai duck (Anas platyrhynchos) and their hybrid (mule duck) used in this study were bred and provided by the Duck Experimental Station, Yeelan, Taiwan. Mitochondrial DNA

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was extracted from livers of freshly sacrificed ducks by the rapid phenol extraction procedure described recently (19).

DNA cloning and hybridization

Hind III fragments of Mascovy mtDNA were cloned into a pBS (Stratagene) vector and transformed into JM 109. Chimeric plasmids of interest were screened, quantitatively prepared, purified, radiolabelled by nick-translation and used as probes to identify overlapping restriction fragments of mtDNA from mascovy and other sources following standard protocols (20). The cloned fragments were also subjected to sequence determination using a sequenase kit (US Biochemical). Sequence homology analysis was done on microVAX II using a GCG program. Corresponding sequences of Xenopus mtDNA kindly provided by Dr. Igor Dawid (21) were used as a control.

Restriction enzyme digestion and physical mapping

Mitochondiral DNA was first subjected to digestion by a bank of common restriction endonucleases in order to select enzymes with a relatively low frequency of cleavage for use in the construction of physical maps, basically by the standard double digestion method (20). Additional enzymes were used to yield digests which were compared electrophoretically.

RESULTS

Restriction patterns

Mitochondrial DNAs from mule duck and its parents were compared electorphoretically upon restriction enzyme digestions. Results with three endonucleases, Bam HI, Hind III and Pst I, show that mule duck is identical to its mother Tsai duck and differ from Mascovy, its father in their restriction patterns. This is illustrated in Figure 1 and suggests that mtDNA in ducks is transmitted maternally.

Physical map

In order to ascertain that mule duck and its mother share an identical mitochondrial genome which is different than Mascovy, a more extensive analysis was made so that their physical map could be compared. In the Mascovy mt genome, there are 1, 1, 2, 2, 2, 6 and 7 sites, respectively, for EcoR I, Bgl II, Bam H1, Pst I, Xba I, Bgl I and Hind III. A circular map is deduced from these results and shown in Figure 2. Most of the endonucleases used cleaved the genome in a narrow region, leaving four Bgl I sites to cover as much as 10 kb of the remaining genome. The order of these four Bgl I sites was determined by hybridization with a 2.3 kb fragment of Hind III digest which covered two Bgl II sites and a Bgl I/Bgl I and a Bgl I/Bgl II

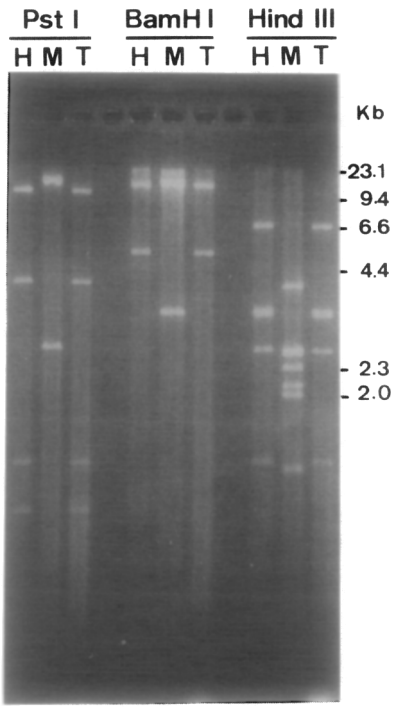

Figure 1. Selected restriction patterns of duck mt genome. Results are presented in three groups from left to right Pst I, Bam H1 and Hind III. Within each group there are three lanes, respectively, from left to right Mule duck (H), Mascovy (M) and Tsai duck (T). Restriction enzyme digests were run on 0.8 % agarose gels in tris-borate-EDTA buffer, pH 8.1 at a constant voltage of approxiamtely 60 volts for 16 hours. DNA fragments were identified by ethidium bromide staining.

junctions (see Fig. 2). Similarly, the physical maps of mule duck and Tsai duck were independently deduced and are also shown in Figure 2.

Location of rDNA and D-loop

By nucleotide sequence determination and computer aided homology anaylysis, a 2.3kb Hind III fragment obtained from Mascovy was shown to code for part of the 16S ribosomal RNA and the D-loop. With this fragment and that of Xenopus as probes, the coding sequences for rDNA and D-loop for Mascovy, Tsai duck and their hybrid are located (Figure 2).

DISCUSSION

Tsai duck is selected for high egg production from Peking duck through generations of outbreeding. No obvious changes could be observed between its mtDNA and that of its original stocks (22). This is interesting in view of the

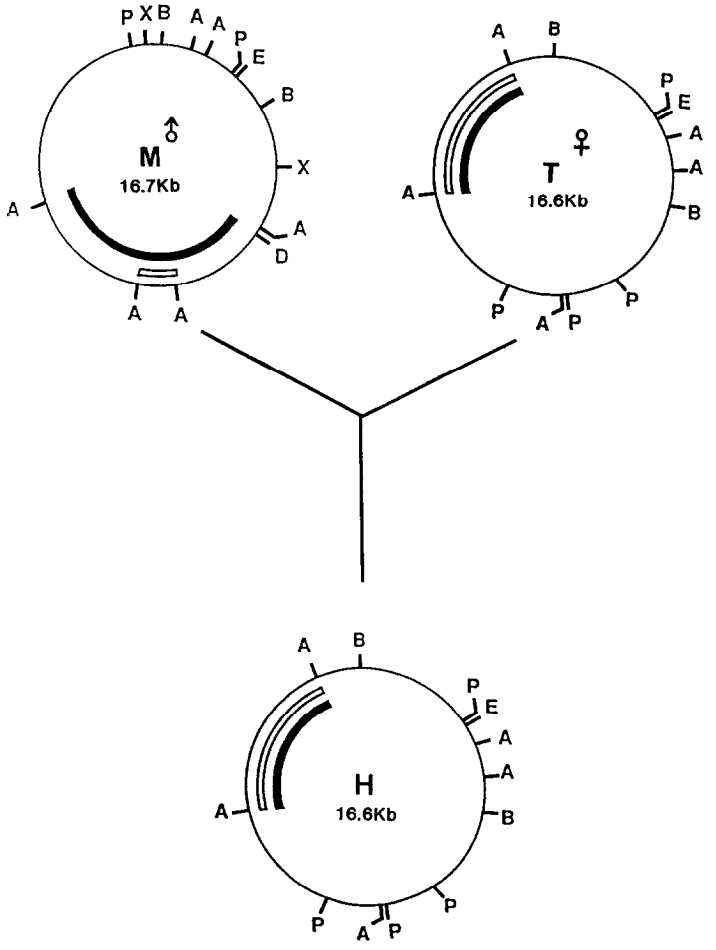

Figure 2. Restriction maps of mtDNAs and their respective locations of D-loop. The three maps were constructed independently using mainly double digestion method and their D-loops located by hybridization using duck (closed bar) or xenopus (open bar) probes following standard protocols. T=Tsai duck; M=mascovy; H=mule duck. For restriction endonulceases A: Bgl I, B: Bam HI, D: Bgl II; E: Eco RI; H: Hind III; P: Pst I, and X: Xbl I.

general tendency for mtDNA to evolve rapidly and limited intraspecific restriction enzyme polymorphism has been noted in chicken mtDNA (15).

Although maternal inheritance of the mitochondrial genome has been suggested in several organisms, it may not be universal. Especially for mammals, preponderance of mitochondria in their eggs but not their sperms may account for the prevalence of maternal mtDNA in zygotes (11). Moreover, in mammals there is the "founder effect", which is the result of oogenic amplification of mtDNA using a very limited number of templates. It remains possible, therefore, that maternal inheritance of mtDNA, in mammals at least,

is a numerical consequence resulted from the high ratio of maternal to paternal mtDNA. This is particularly biased as mitochondria of the single sperm that enters the egg generally disintegrate upon fertilization.

Vertebrates with large ova, such as the birds, differ from mammals in that several spermatozoa may enter the egg. Polyspermy is noted in pigeons; during fertilization 12 to 25 sperm are involved per egg (23). Although only one of these sperm will fuse with the egg nucleus, the remaining supernumerary sperm nuclei migrate to the blastodisc where they become temporarily active, dividing and furnishing a secondary area of small cells by accessory cleavage. These small cells surround "the true cleavage cells produced by division of the central portion of the disc around the descendants of the segmental nucleus" (23).

Furthermore, in contrast to isolecithal ova of mammals, which essentially devoid of yolk, telolecithal ova of birds contain a large amount of yolk. Fetal development in birds consumes the yolk during metamorphosis and fewer mitochondria are expected to survive. The surviving mtDNA undergoes little rearrangement in birds, unlike that in other animals whose mtDNA exhibits extensive intraspecific polymorphism of restriction sites (16). Taken together, its seems reasonable to speculate that if paternal endowment contributes to the passage of mtDNA, it should be observed more readily in the ovaripirous birds. It is thus significant that we are able to demonstrate in this study by restriction endonuclease mapping that mtDNA in two different genera of ducks and their intergeneric hybrid is maternally transmitted.

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REFERENCES

1. Dawid, I.B. and Blackler, A.W. (1972) Dev. Biol. 29, 152-161.
2. Desalle, R., Giddings, L.V. and Templeton, A.R. (1986) Heredity 56, 75-85.
3. Reilly, J.G. and Thomas, C.A., Jr. (1980) Plasmid 3, 109-115.

4. MacNell, D., and Strobeck, C. (1987) Evolution 41, 873-881.
5. Takahiro, Y. et al. (1987) Biochem. Genet. 25, 429-446.
6. Ashley, M. and Wills, O.C. (1987) Evolution 41, 854-863.
7. Francisco, J.F., Brown, G.G. and Simpson, M.V. (1979) Plasmid 2, 426-436.
8. Hayashi, J.T., Yonegawa, H., Gotoh, O., Watanabe, J. and Tagashira, Y. (1978) Biochem. Biophys. Res. Commun. 83, 1032-1038.
9. Kroon, A.M., deVos, W.M. and Bakker, H. (1978) Biochem. Biophys. Acta 519, 269-273.
10. Nelson, K., Baber, R.J. and Honeycutt, R. (1987) Evolution 41, 864-872.
11. Hutchison, C.A., III, Newbold, J.E., Potter, S.S. and Edgell, M.H. (1974) Nature 251, 536-538.
12. Giles, R.E., Stroynowski, I. and Wallace, D.C. (1980) Somat. Cell Genet. 6, 543-554.
13. Horai, S., Gojobori, T. and Matsunaga, E. (1986) Jap. J. Genet. 61, 271-275.
14. Cann, R., Stoneking, M. and Wilson, A.C. (1987) Nature 325, 31-36.
15. Glaus, K.L.(1980) Ph.D. Dissertation, Ohio State University. 319pp.
16. Ball, R.M. et al. (1988) Proc. Natl. Acad. Sci. USA 85, 1558-1562.
17. Kessler, L.G. and Avise, J.C. (1984) Sys. Zool. 33, 370-380.
18. Shields, G.F. and Wilson, A.C. (1987) Evolution 41, 662-666.
19. Tzeng, C.S., Shen, S.C. and Huang, P.C. (1990) Bull. Inst. Zool. Acad. Sinica 29, 11-19.
20. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) MOLECULAR CLONING, A LABORATORY MANUAL. Cold Spring Harbor Laboratory, NY. 545pp.
21. Rastl,E. and Dawid,I.B. (1979) Cell 18, 501-510.
22. Zhao, B., Xu, H., Ma, S., Li, L. and Shen, T (1983) Sci. Sinica(B) 26, 1143-1154.
23. Hamilton, H.L. (1953) Lillie's DEVELOPMENT OF THE CHICK. Henry Holt & Co., NY, 306pp.