Transmission and Segregation of mtDNA

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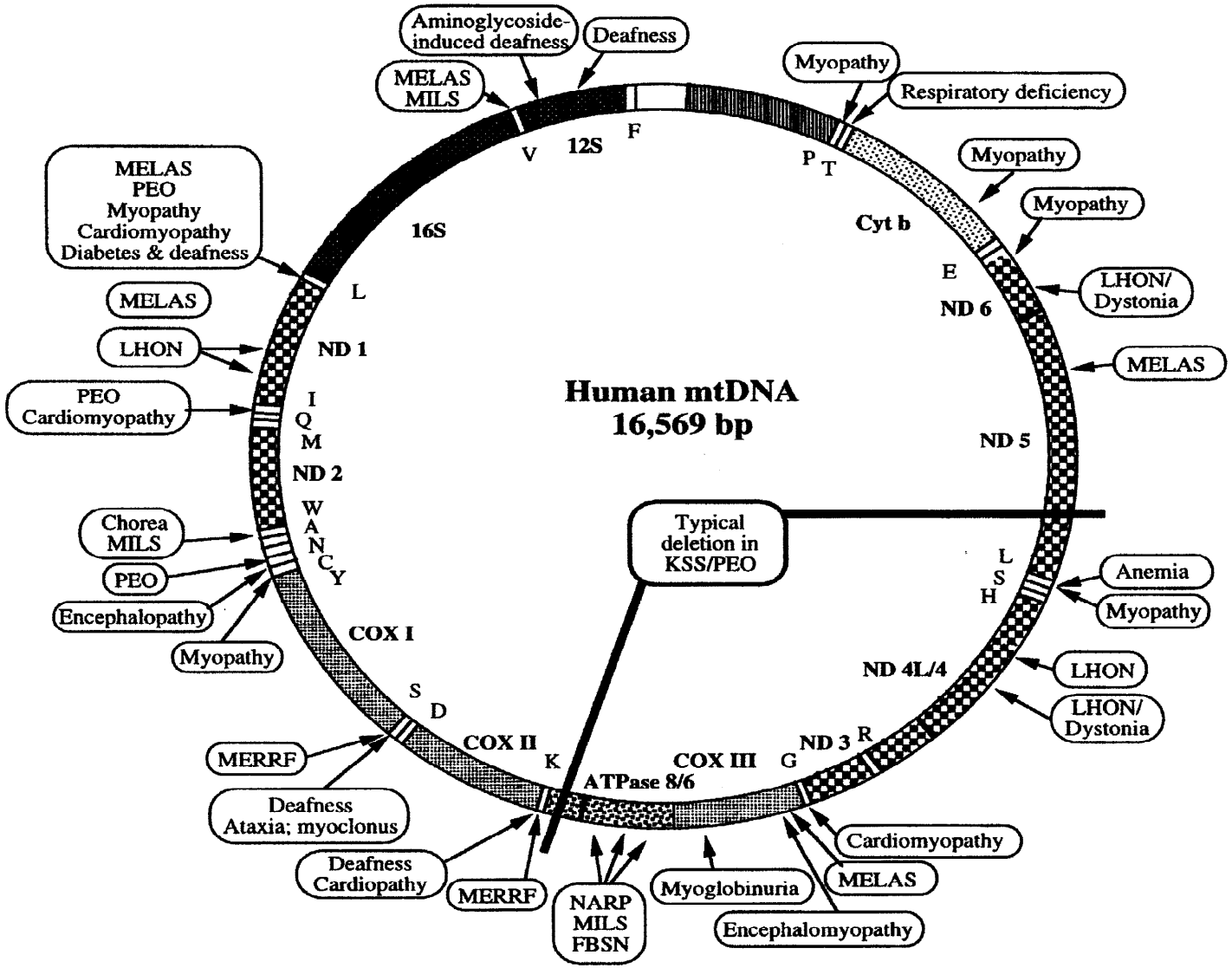
Mitochondrial Genetics

- Thousand copy genome, maternally inherited
- Germline and somatic mutations produce mtDNA heteroplasmy
- Replication not tightly linked to cell cycle
- Random partitioning at cytokinesis
- Replicative segregation


Human mtDNA
16,569 bp
Aminoglycoside-induced deafness
Deafness
MELAS
MILS
Myopathy
Respiratory deficiency
Myopathy
Myopathy
MELAS
PEO
Myopathy
Cardiomyopathy
Diabetes & deafness
MELAS
LHON
PEO
Cardiomyopathy
Chorea
MILS
PEO
Encephalopathy
Myopathy
MERRF
Deafness
Ataxia; myoclonus
Deafness
Cardiopathy
MERRF
NARP
MILS
FBSN
Myoglobinuria
Encephalomyopathy
Cardiomyopathy
MELAS
LHON/
Dystonia
MELAS
Anemia
Myopathy
LHON
LHON/
Dystonia
Typical
deletion in
KSS/PEO
12S
16S
Cyt b
ND 6
ND 5
ND 4L/4
ND 3
COX III
ATPase 8/6
COX II
COX I
ND 2
ND 1
F
V
P
T
E
L
I
Q
M
W
A
N
C
Y
S
D
K
G
R
H
S
L

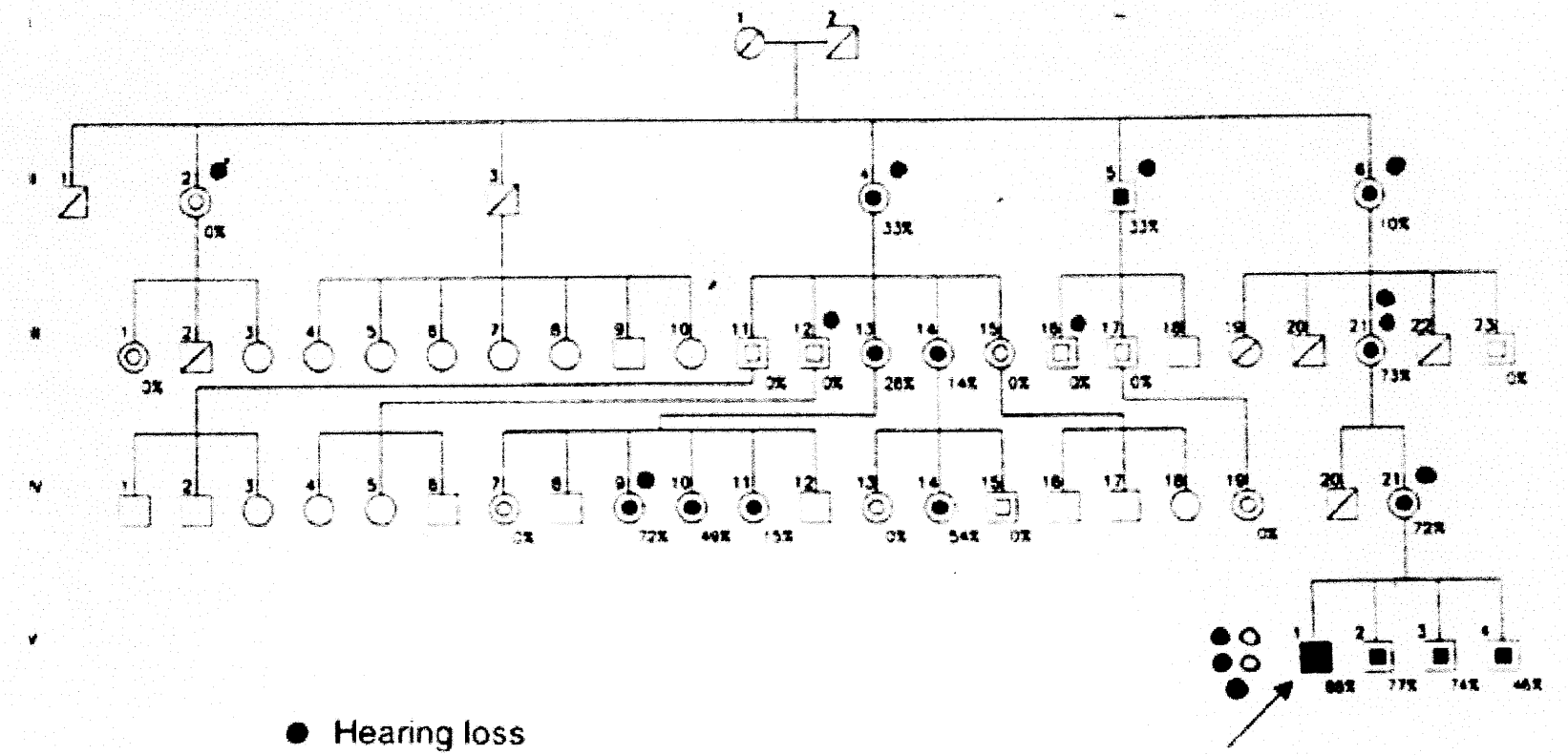
Questions

- How is mtDNA transmitted between generations?

- What controls the segregation of mtDNA sequence variants?

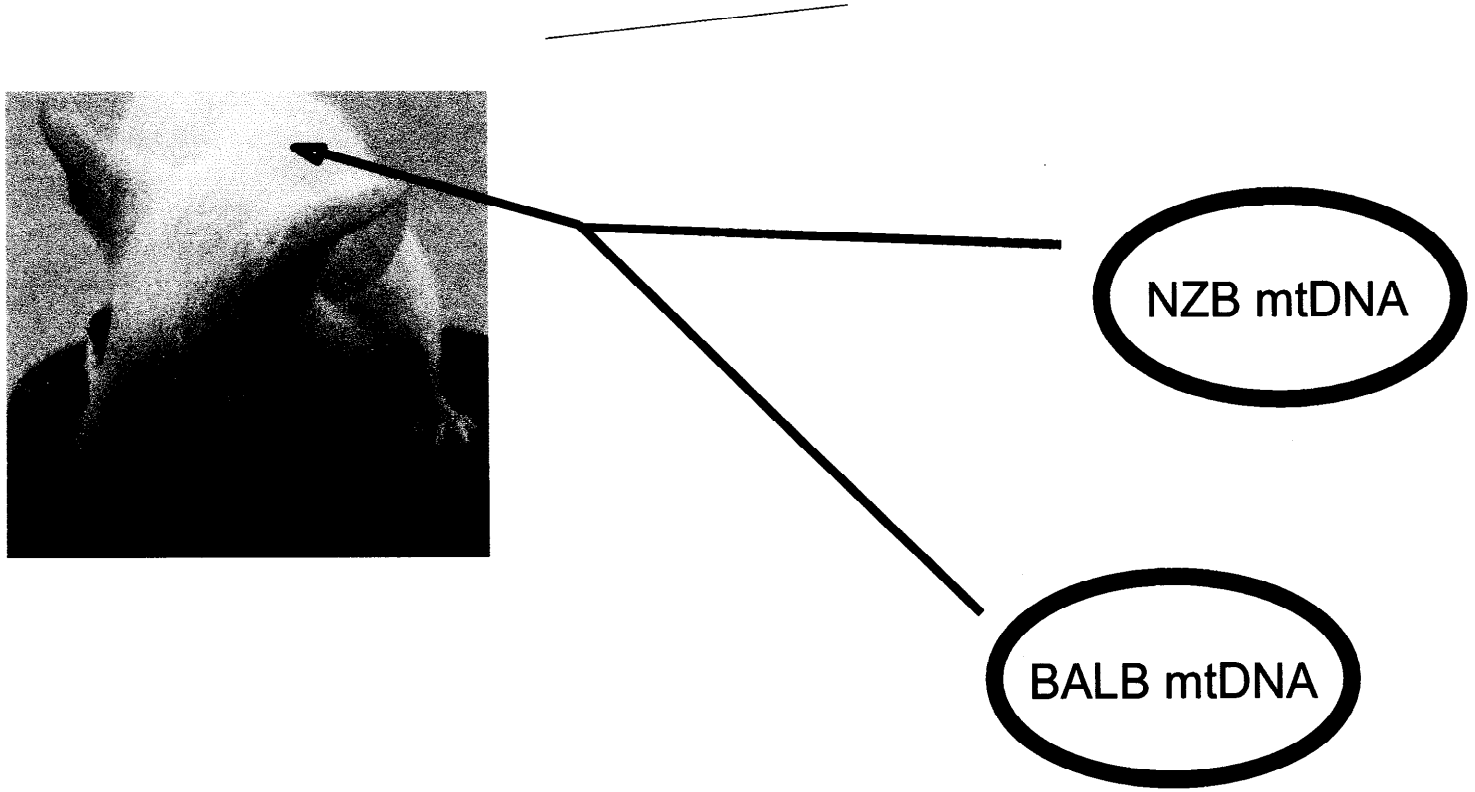
mtDNA Segregates Rapidly in Human Pedigrees

Segregation and Clinical Features of the A8344G tRNA^{Lys} Mutation in a MERRF Pedigree



- Hearing loss
- Myoclonus
- Ataxia
- Lipoma
- Dementia
- Myopathy

Mouse Model of mtDNA Segregation



Generation of heteroplasmic mice

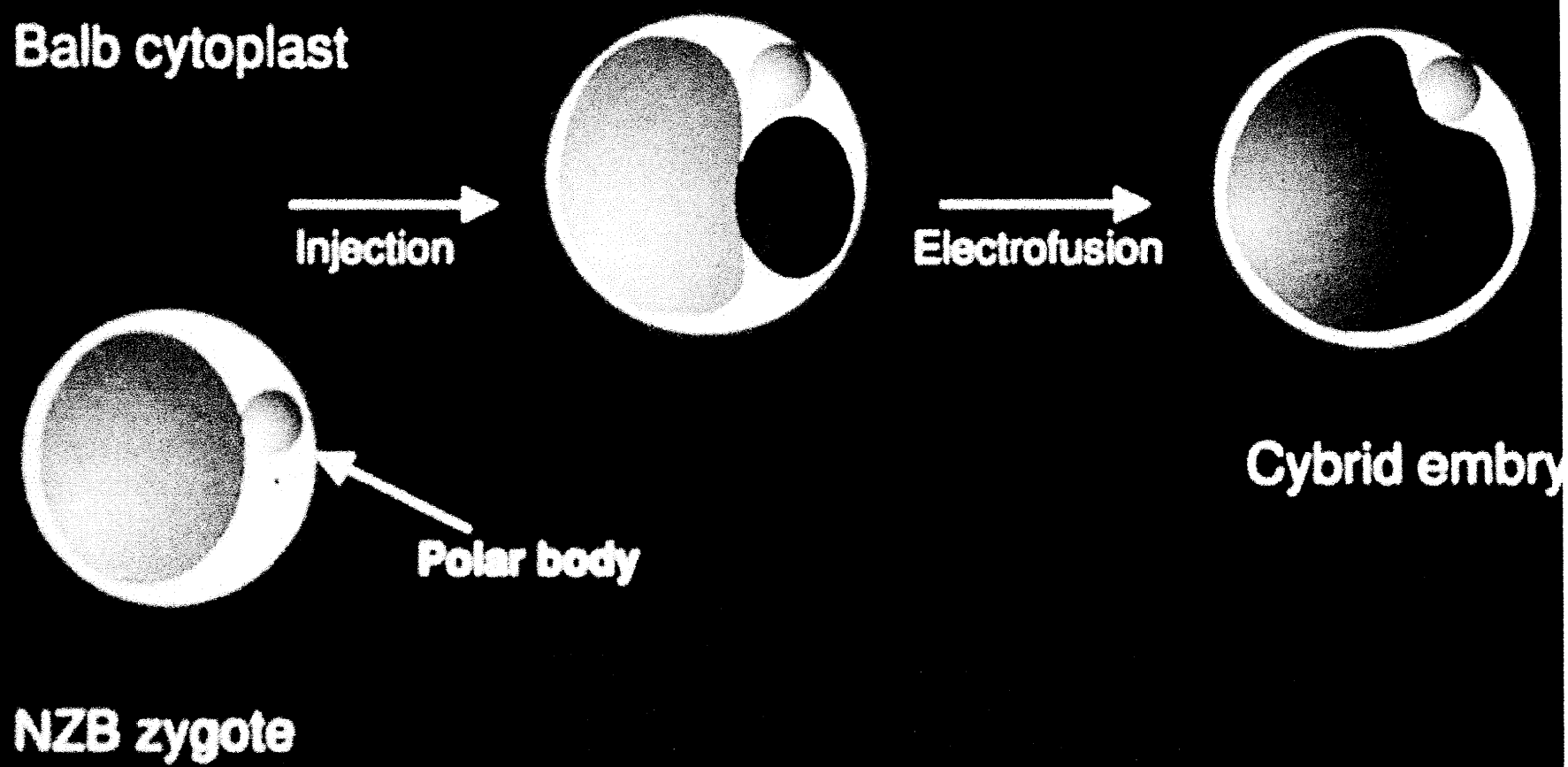
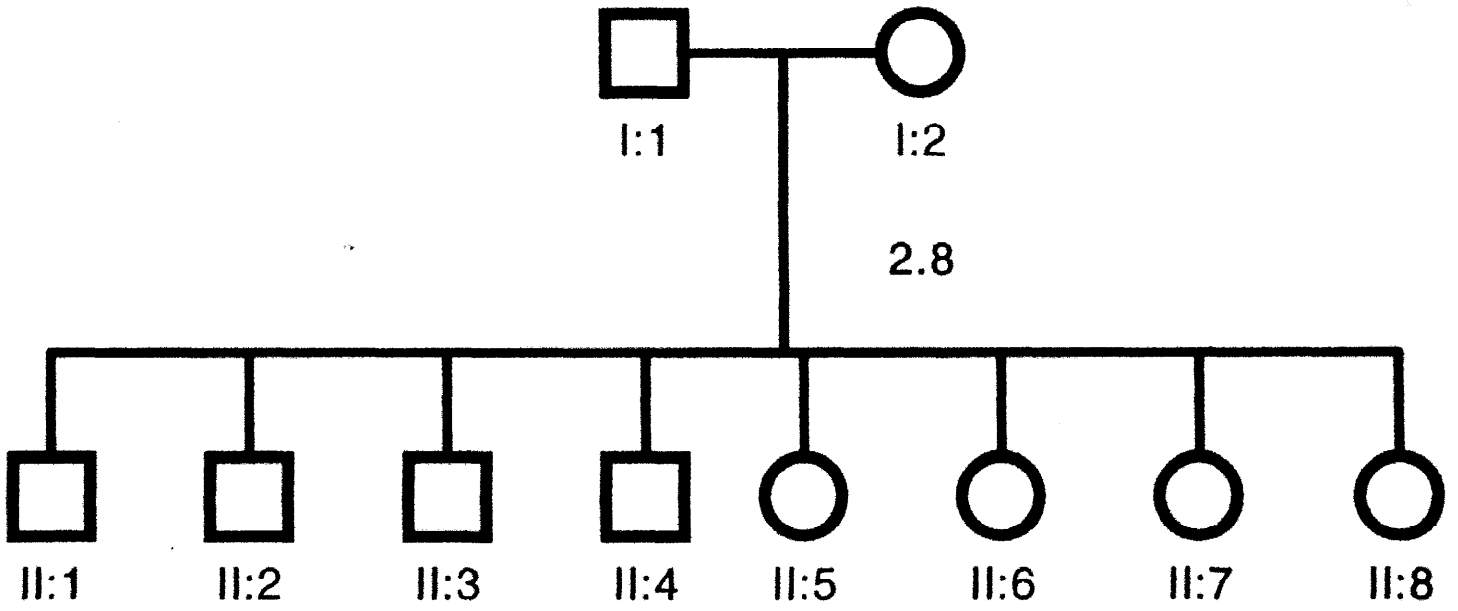


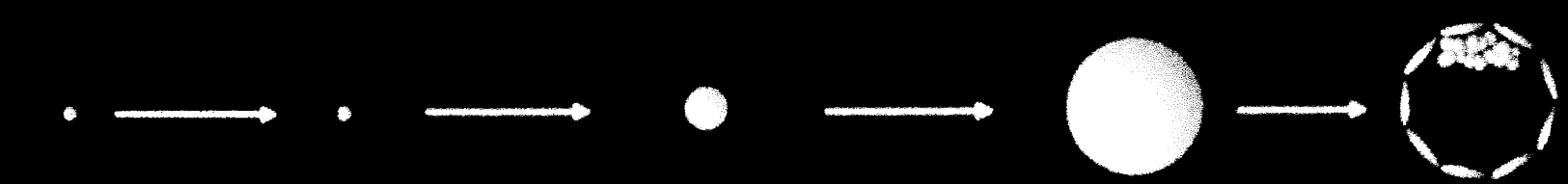
Table 1: Amino acid differences in the mtDNA encoded polypeptides of NZB and BALB mice compared to other vertebrate species

		Mouse						
	AA[a]	NZB	C3H /AN	Rat	Gorilla	Human	Frog	Trout
Complex I								
ND1	3	A	I	I	L	L	P	P
	59	C	R	R	K	K	R	R
ND2	7	T	A	T	P	P	S	T
	265	V	I	A	L	A	F	L
	294	T	I	T	L	L	L	L
ND4	263	M	I	I	L	L	I	L
ND4L	37	M	V	M	M	M	L	M
ND5	103	F	L	F	F	F	F	F
	365	T	I	A	A	T	S	L
	568	I	T	I	Q	Q	N	Q
ND6	91	A	I	F	A	A	I	V
	122	I	V	I	G	G	G	G
Complex III								
Cyt b	24	T	A	A	T	T	T	A
Complex IV								
CoxI	46	T	A	A	N	N	T	A
CoxIII	248	V	I	V	V	V	V	V

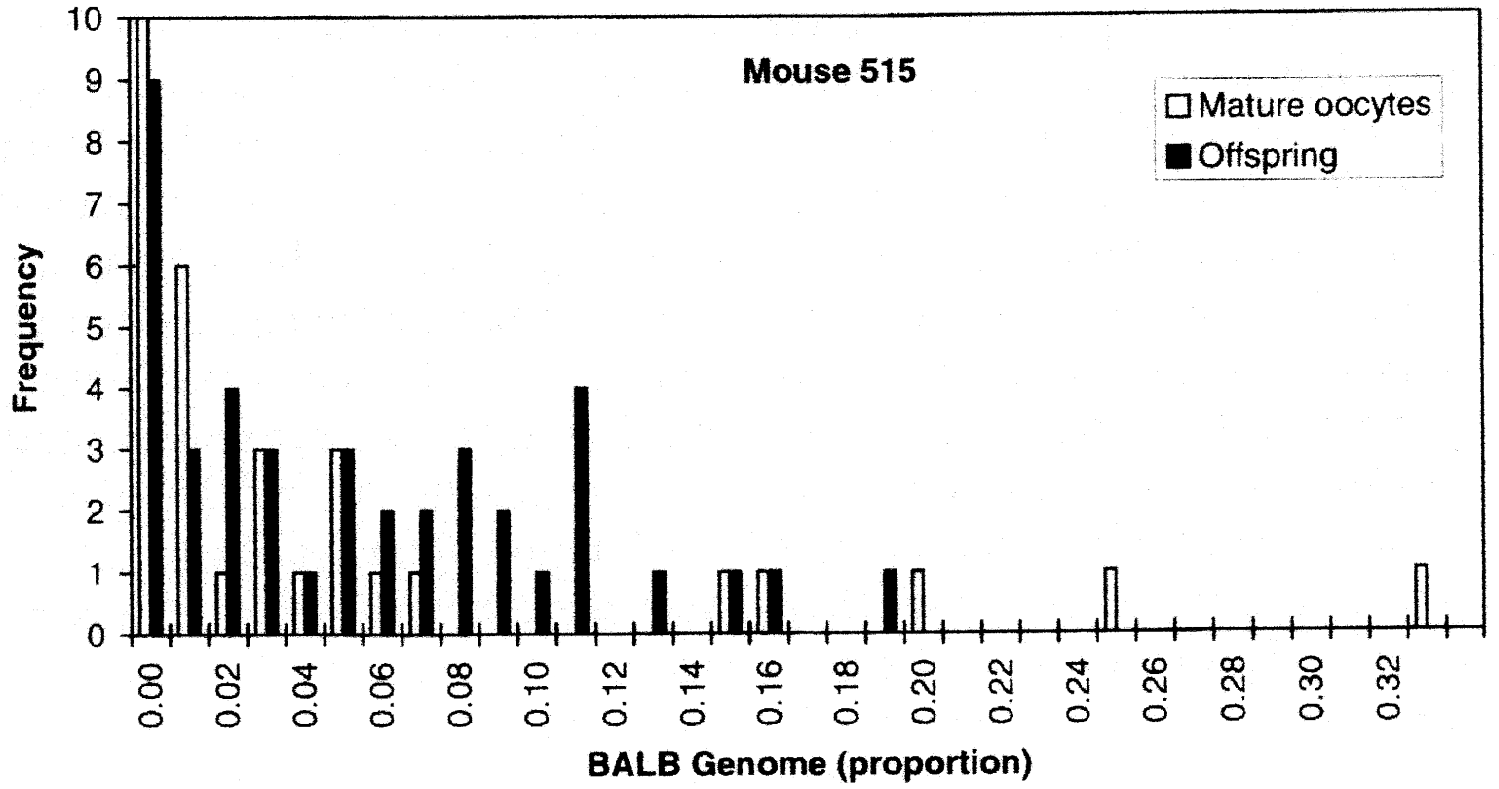
Transmission of NZB mtDNA from a founder heteroplasmic female



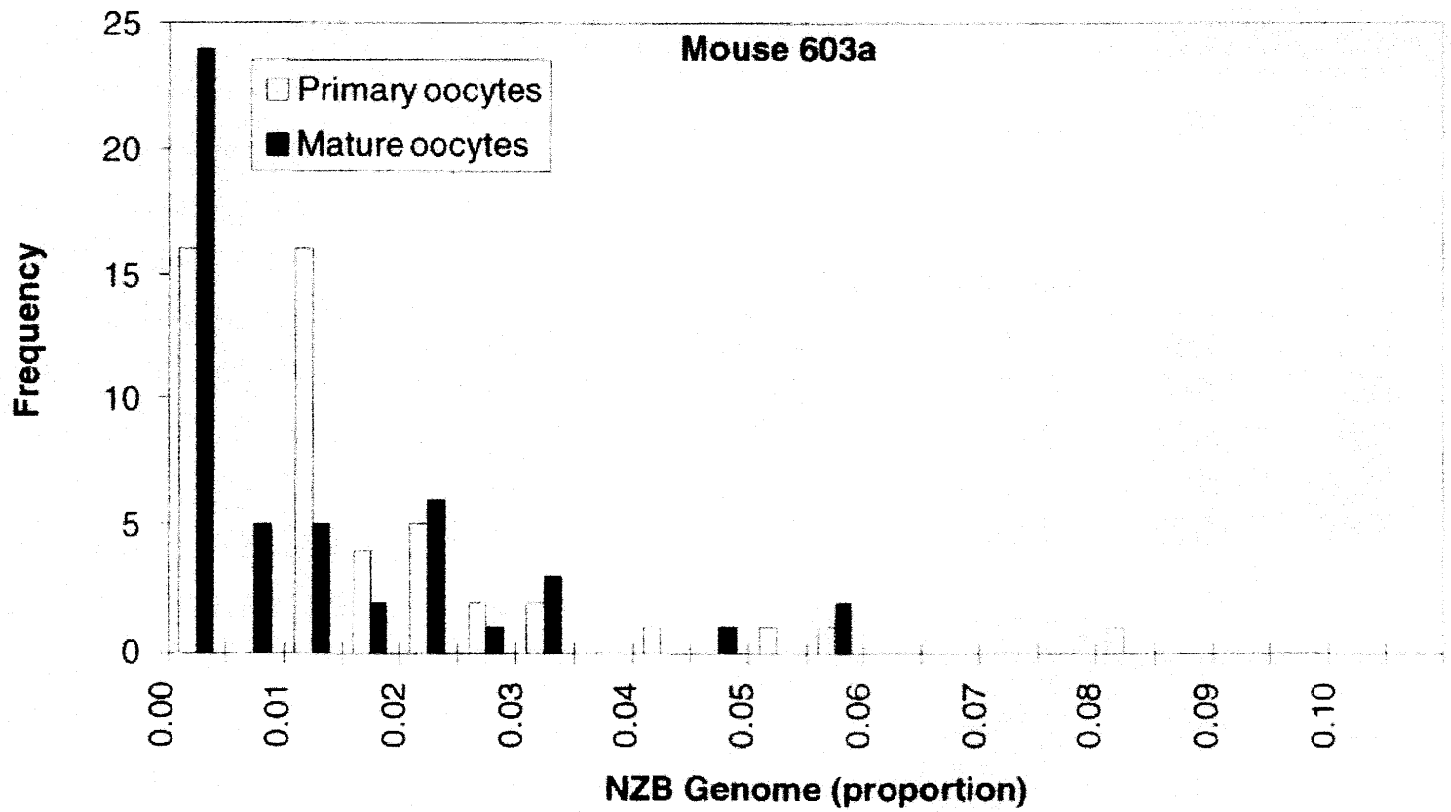
Oogenesis and early development



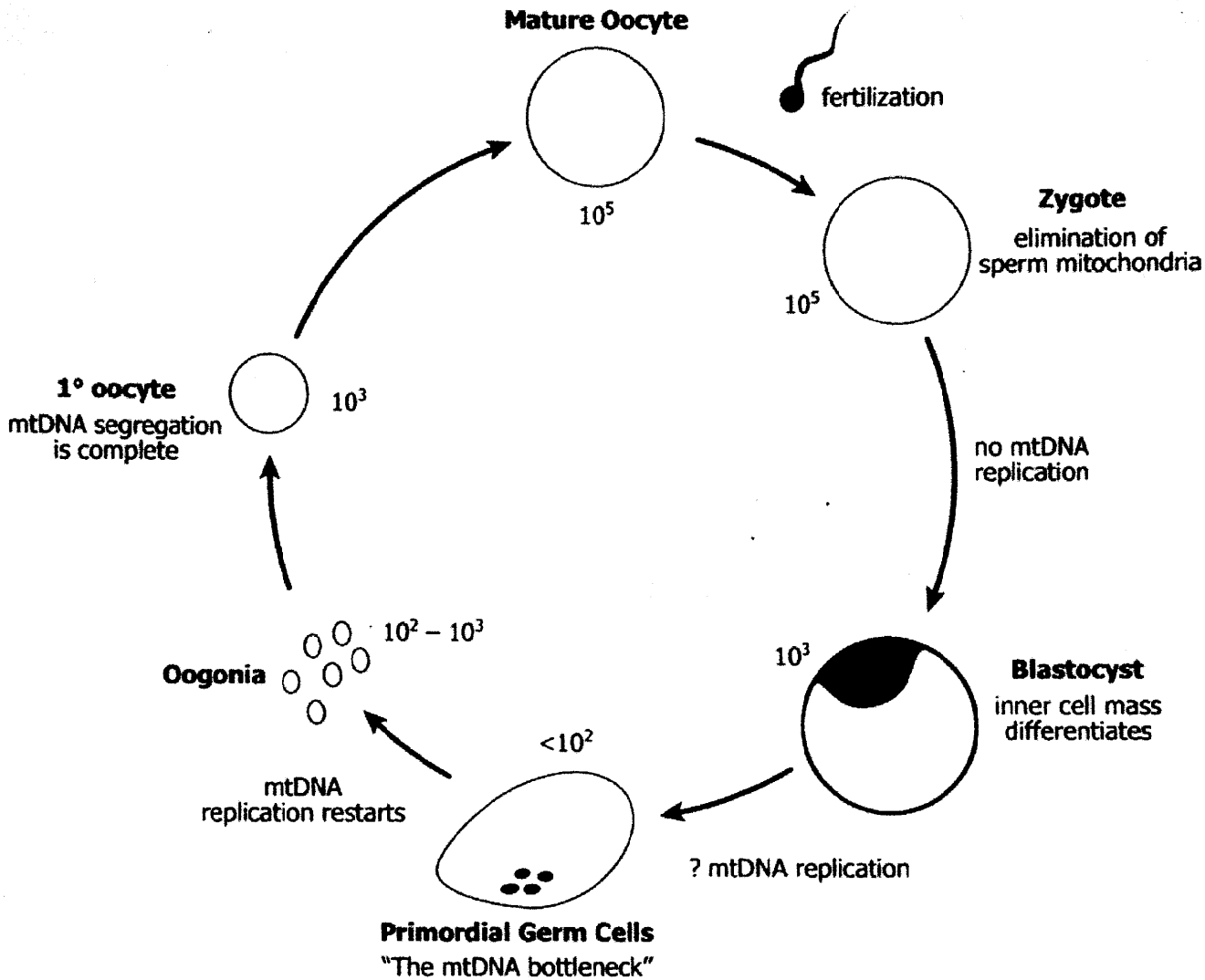
No segregation between mature oocytes and offspring



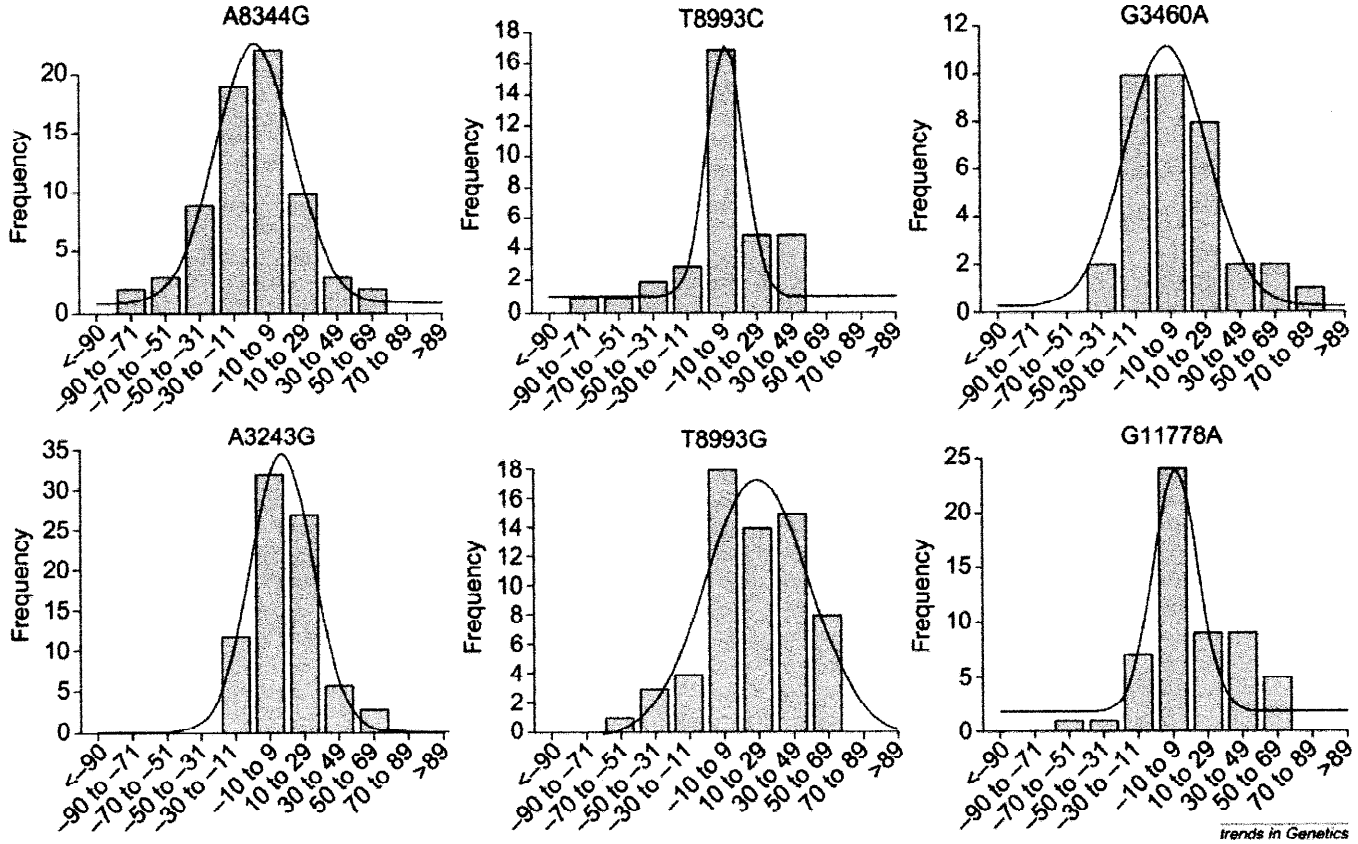
No segregation between primary and mature oocytes



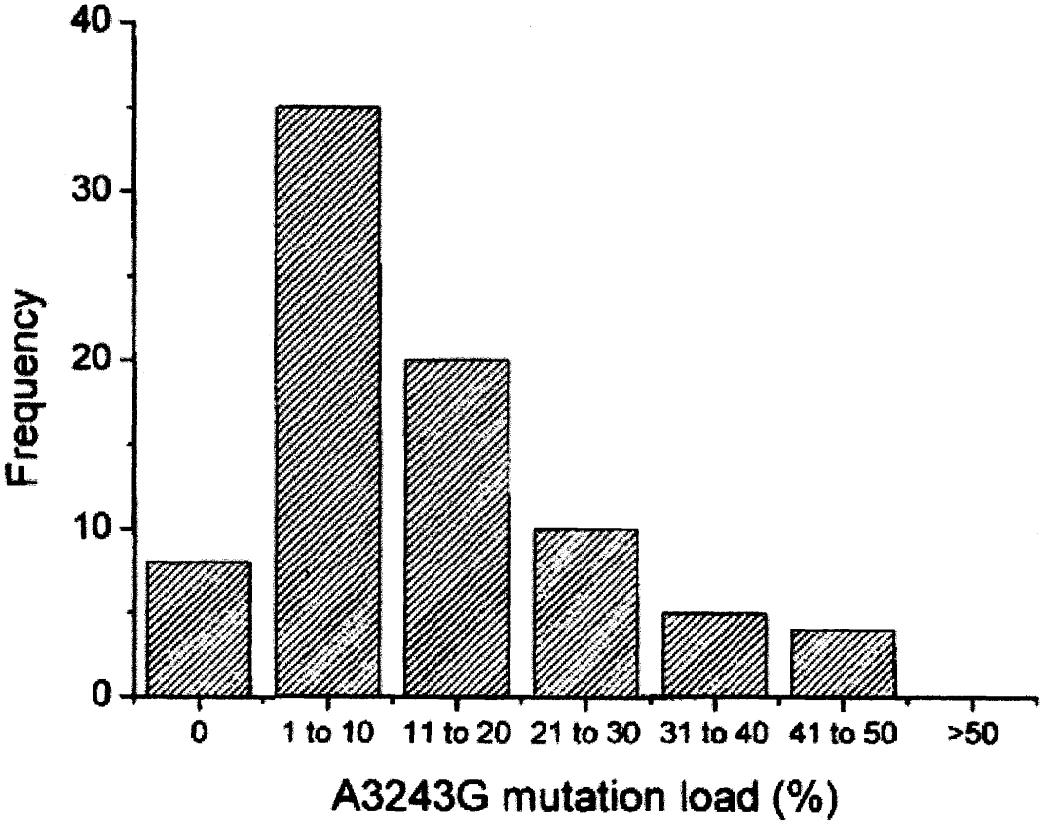
Life Cycle of mtDNA



Transmission of Pathogenic Mutations



Distribution of Heteroplasmy in Oocytes



Conclusions

- Transmission of mtDNA is primarily stochastic
- Effective number of mtDNAs in the germline is small
- Bottleneck causes rapid segregation of sequence variants
- No strong selection for mitochondrial function

mtDNA Segregation - Human Disease

Large scale deletions:

- increase with age in post-mitotic tissues
- decrease with age in the blood

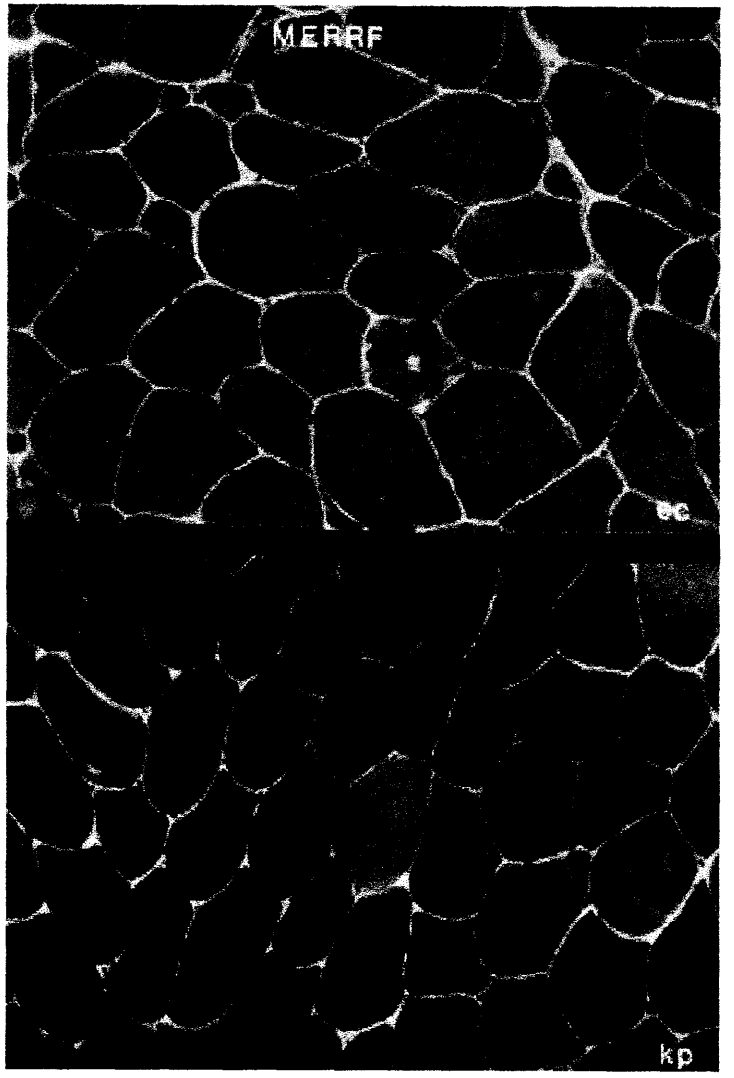
tRNA point mutations:

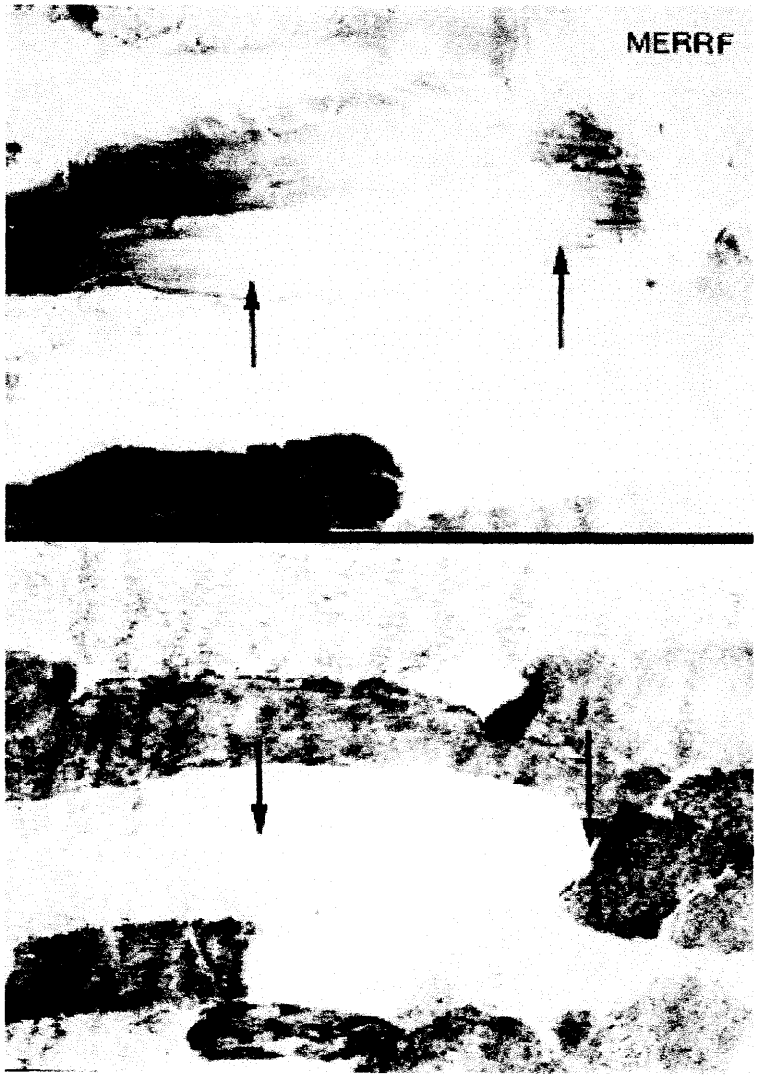
- increase with age in skeletal muscle, undetectable in blood and satellite cells
  (G12315A & A12320G) $tRNA^{Leu(CUN)}$

- no change with age in skeletal muscle, high proportion in blood
  (A8344G) $tRNA^{Lys}$

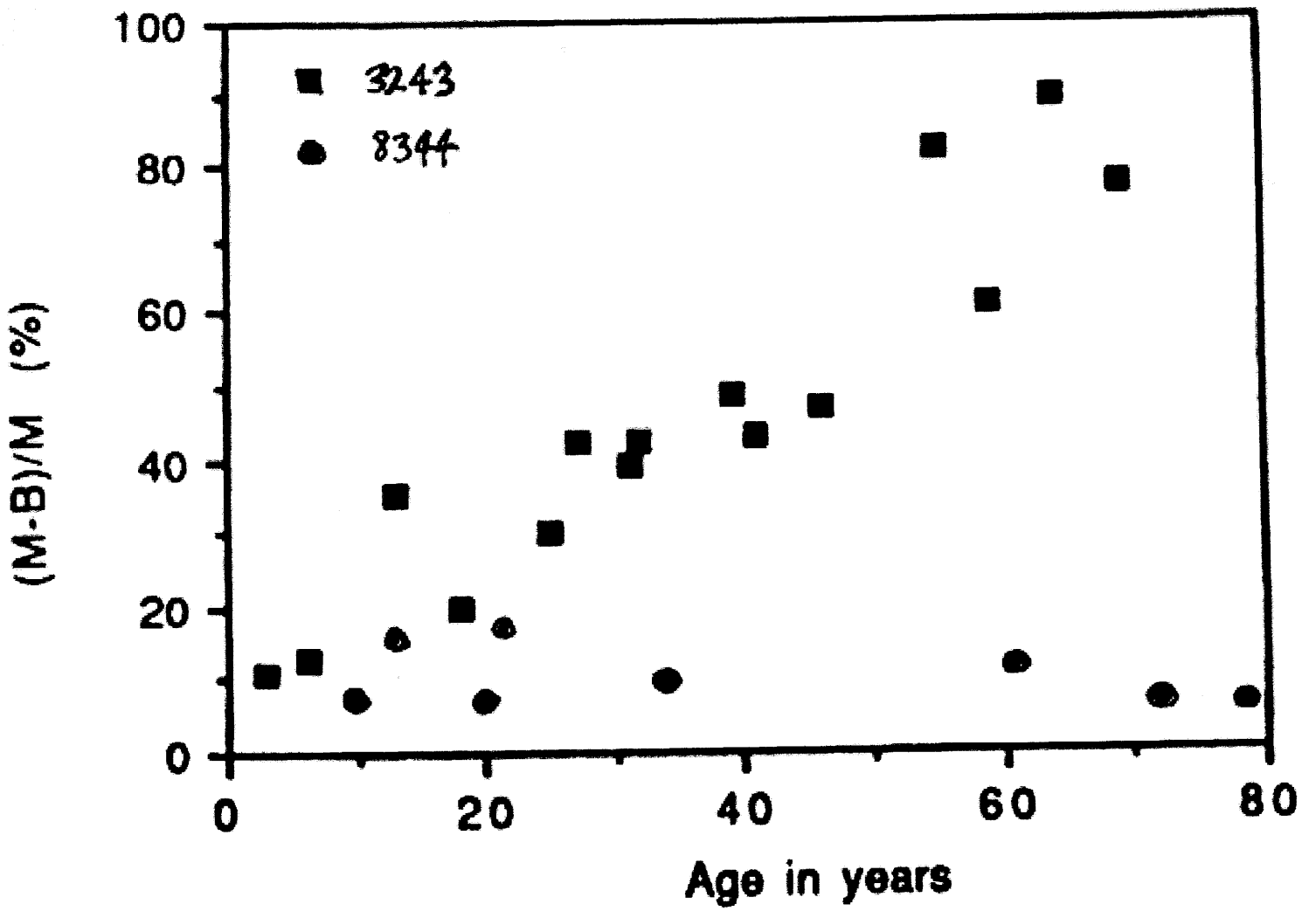
- decrease with age in blood, high proportion in epithelial cells
  (A3243G) $tRNA^{Leu(UUR)}$

Segregation of pathogenic mtDNAs determines the muscle phenotype





Different segregation patterns associated with two tRNA point mutations



Conclusion

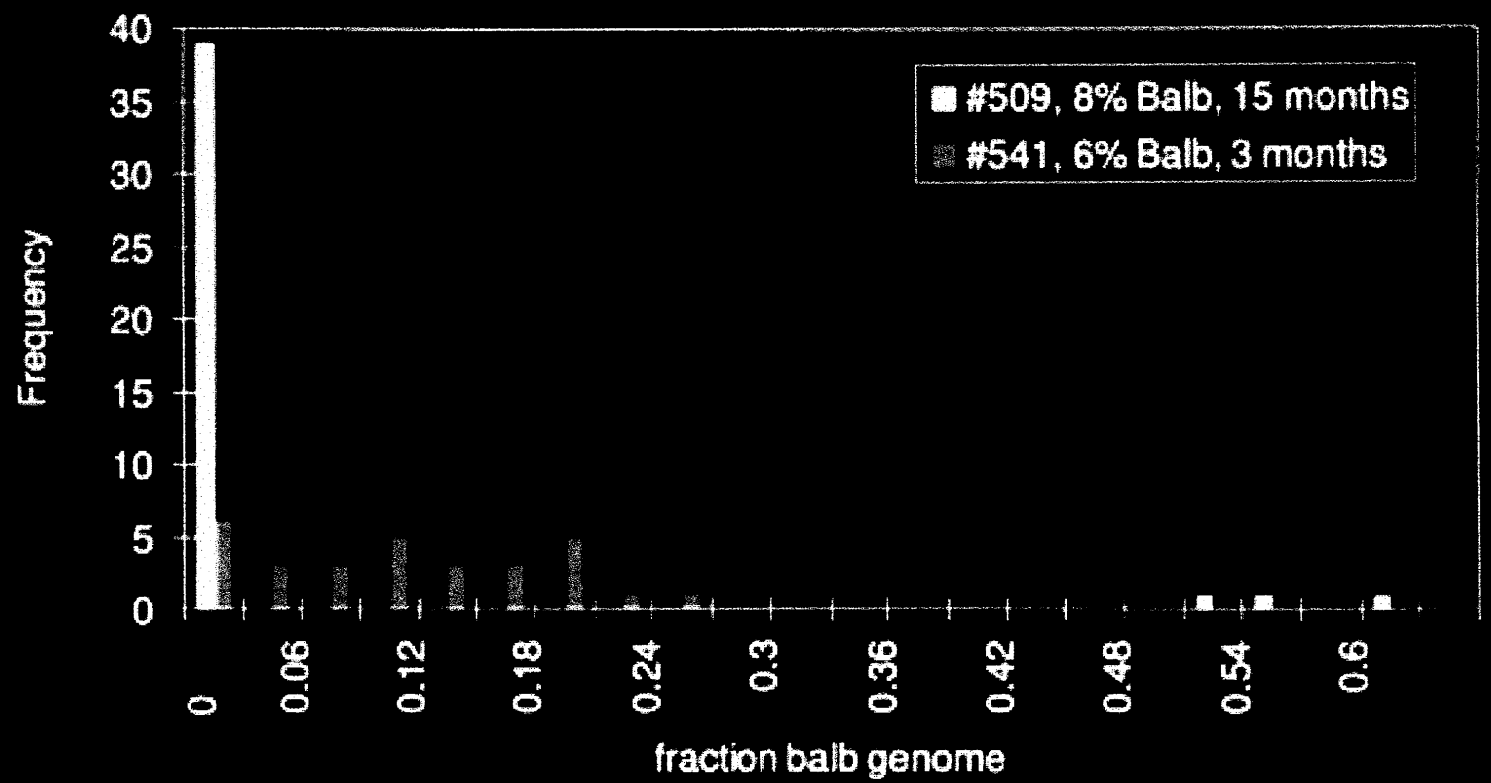
There is no simple relationship between oxidative phosphorylation dysfunction and the pattern of mtDNA segregation

Mouse Model of mtDNA Segregation

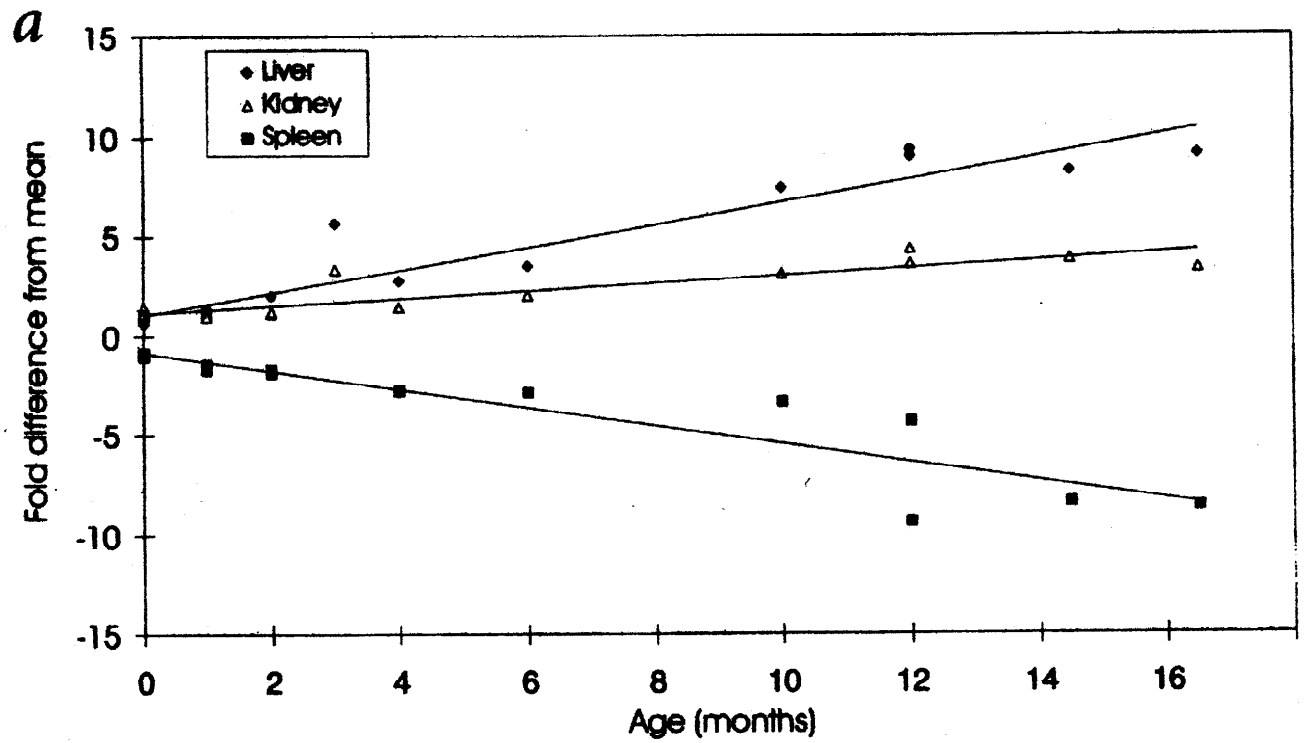
NZB mtDNA

BALB mtDNA

Segregation of colonic crypts



Tissue-specific and age-dependent selection



Mechanism???

Selection at the level of the cell, organelle, genome???

NZB mtDNA Selection *In vivo* in Hepatocytes

Relative Fitness
(advantage of mtDNA)

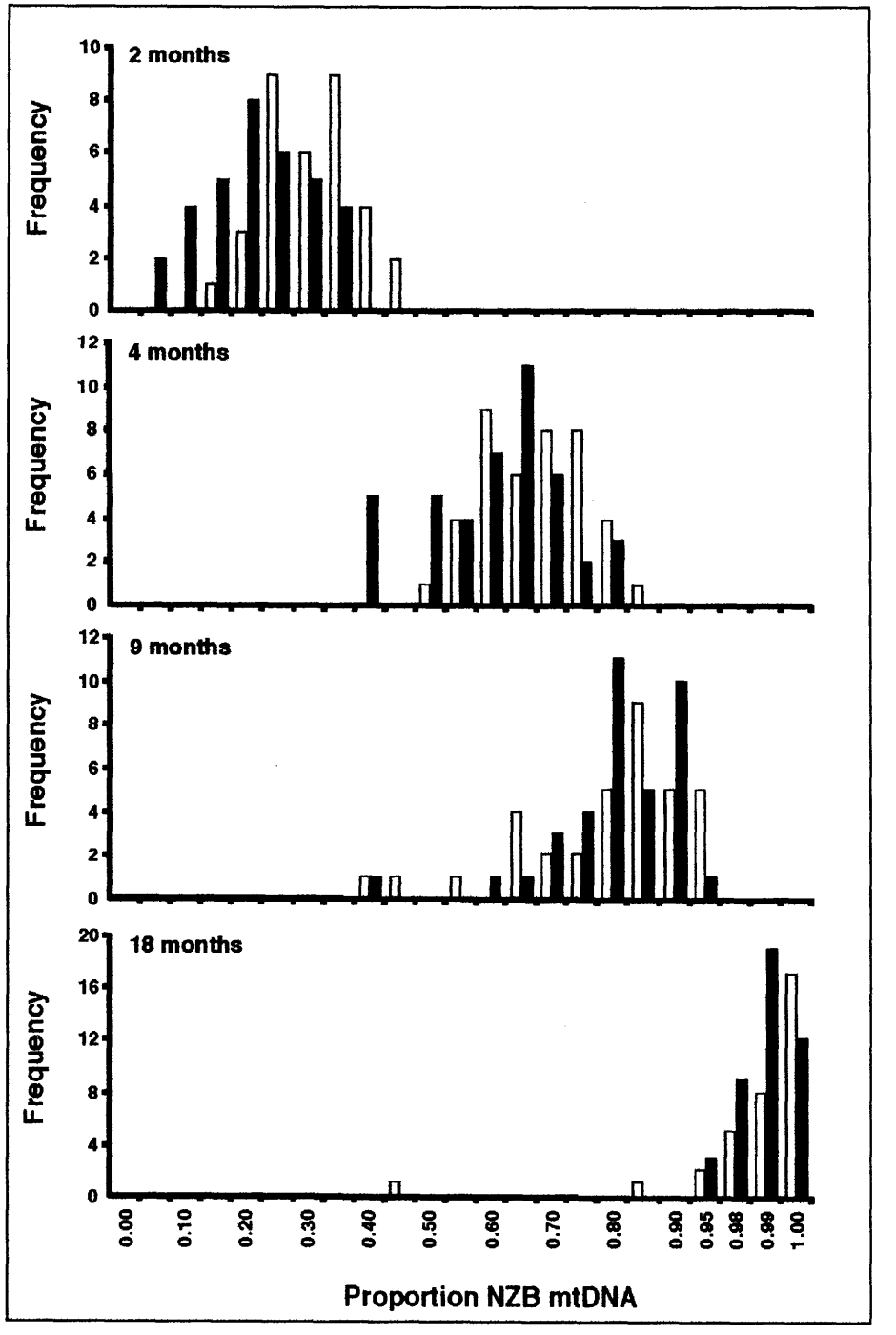
relative fitness

turnover

$$\left(\frac{p_n}{q_n}\right) = \left(\frac{w}{w1}\right)^n \left(\frac{p_0}{q_0}\right)$$

final frequency

initial frequency



NZB mtDNA Selection *In vivo* in Hepatocytes

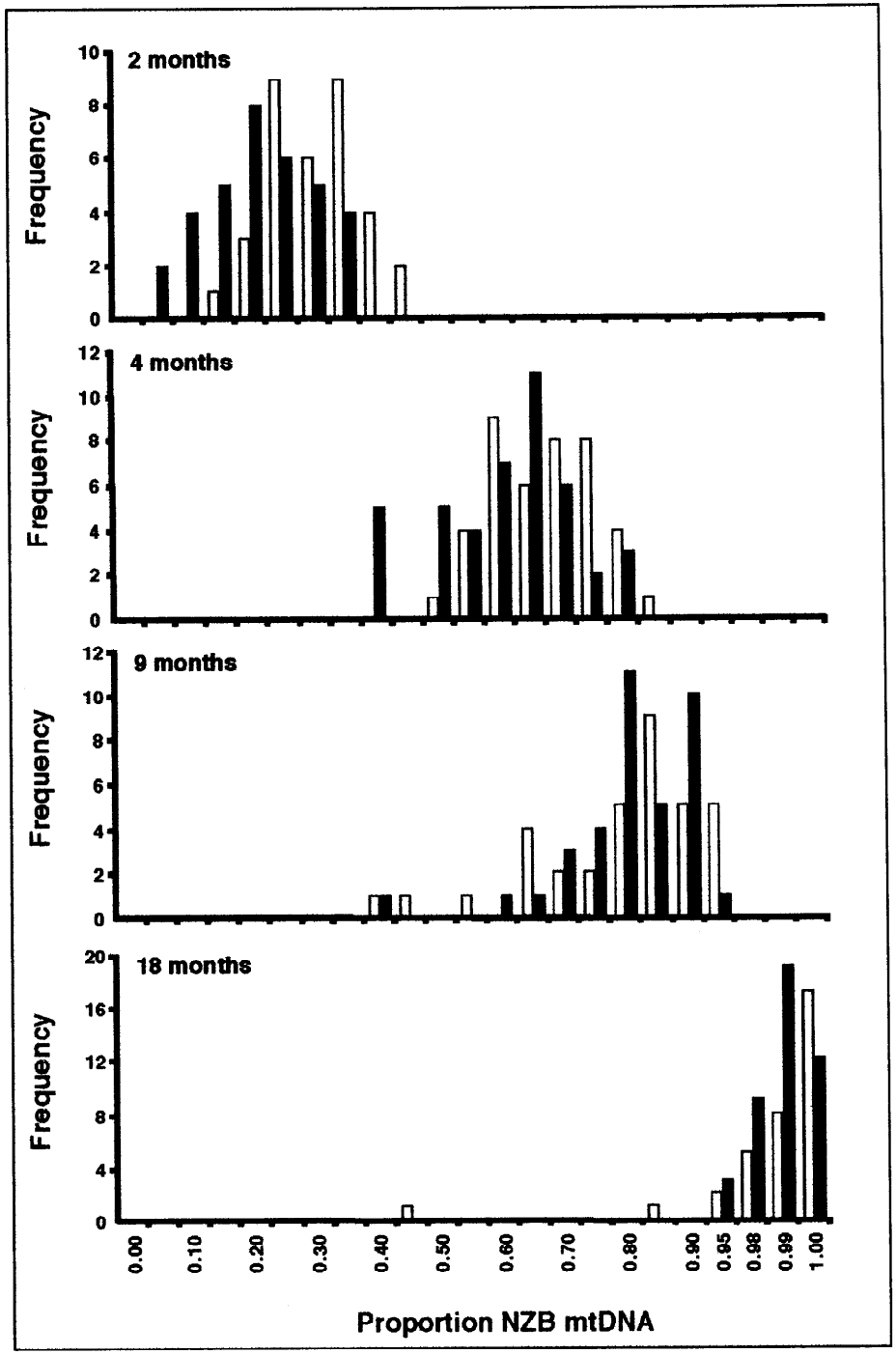
Relative Fitness
turnover 9.4 days

2 mths
1.13 (0.03)

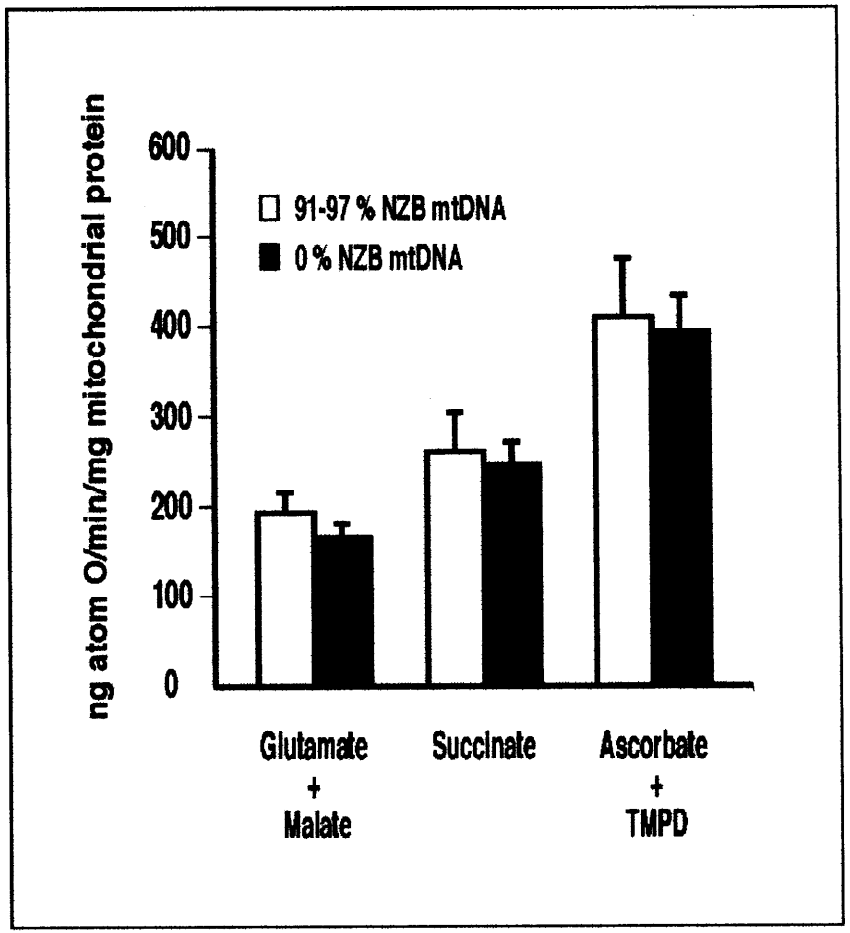
4 mths
1.16 (0.01)

9 mths
1.12 (0.01)

Constant 14 % advantage for NZB mtDNA in hepatocytes

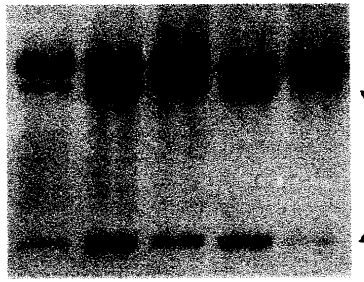


Mitochondrial Oxygen Consumption



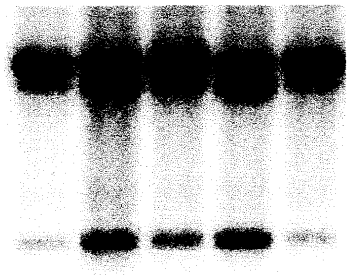
In vivo mtDNA Replication

BrdU detection



%NZB 83 47 74 42 88

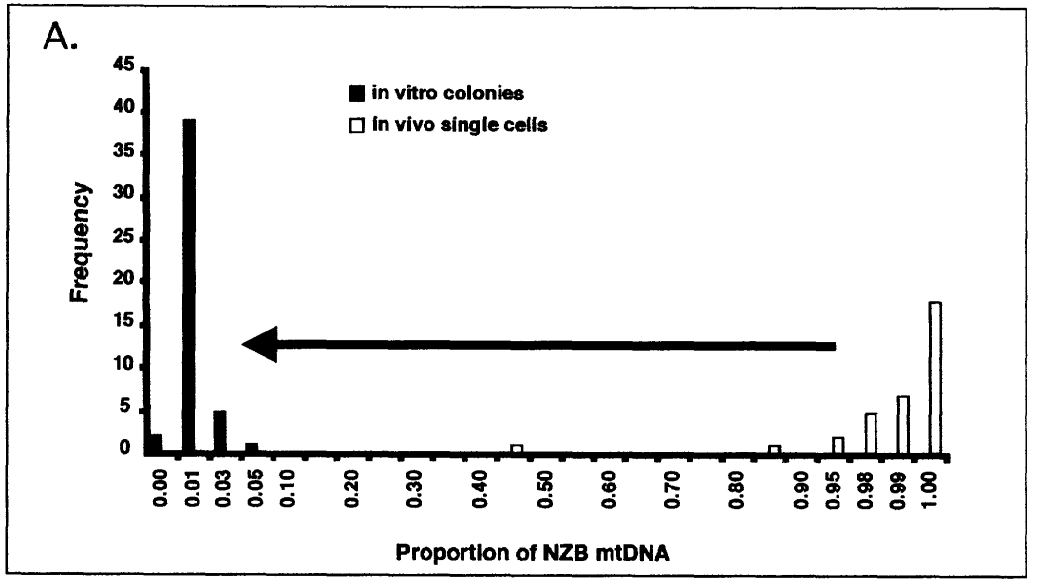
Southern



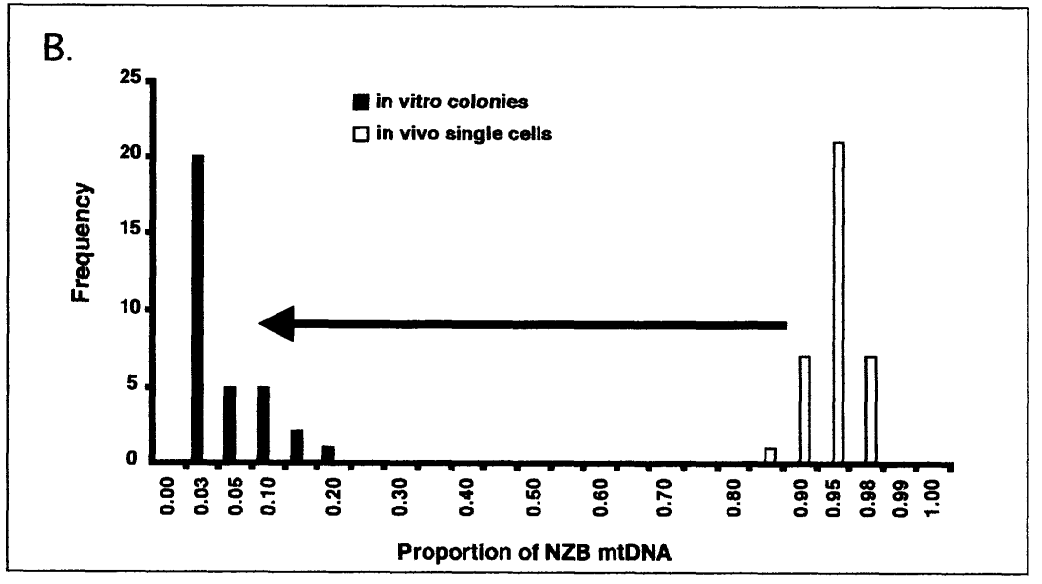
% NZB 83 48 70 39 86

BALB mtDNA Selection *In Vitro*

18 mth



9 mth



In Vitro Relative Fitness for BALB mtDNA

32%

NZB mtDNA Selection in Liver

- 14% advantage for NZB mtDNA, independent of genotype frequency
- Respiratory chain function not involved in selection
- mtDNA replication cannot account for advantage
- Mode of growth can determine genotypic selection

Selection at the level of the mitochondrial genome

Mouse Breeding Strategy for Linkage Analysis

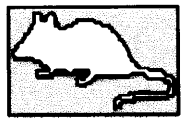
PARENTALS

nDNA : BALB

mtDNA : BALB
NZB

female

X



male

nDNA : CAST

mtDNA : CAST

select NZB mtDNA in liver

F_1

nDNA : BALB / CAST

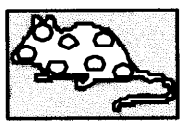
mtDNA : BALB
NZB

sister **X** brother

F_2

nDNA : BALB / CAST (1:2:1)

mtDNA : BALB
NZB



collect tissues at 3 and 12 months

measure % NZB heteroplasmy

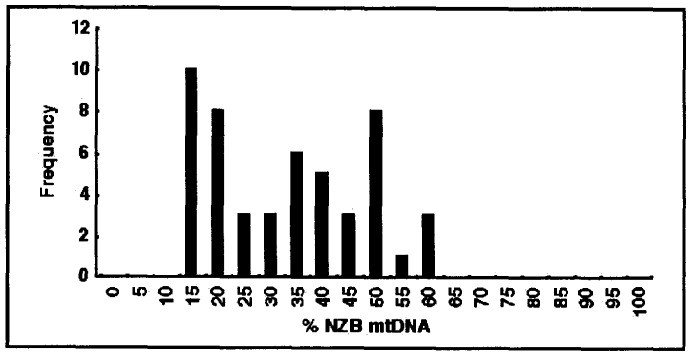
?? select NZB mtDNA in liver ??

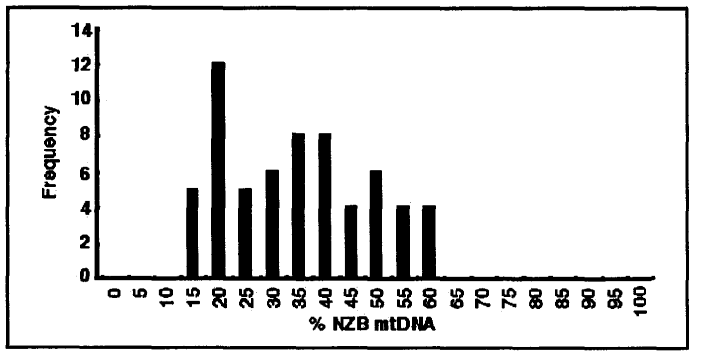
NZB mtDNA Segregation in F2 Mice with Age

3 months

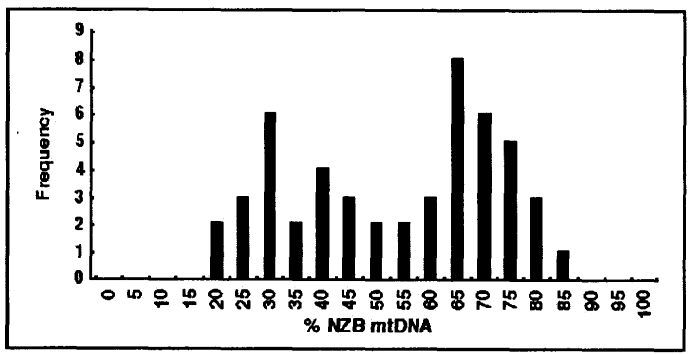
Muscle

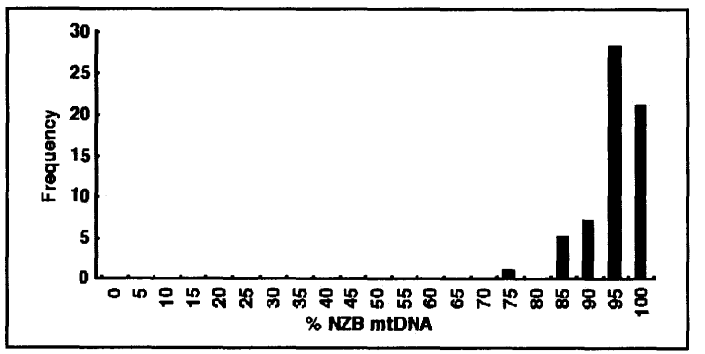
12 months





Liver



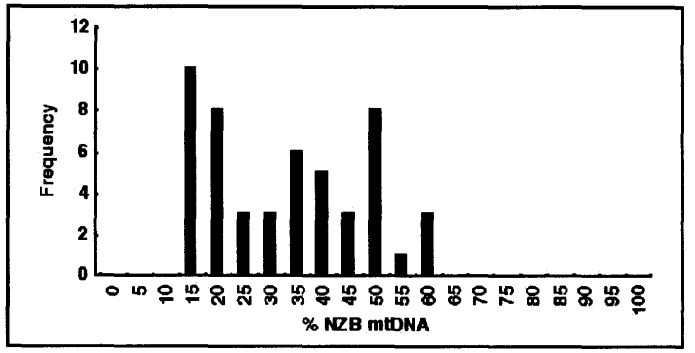


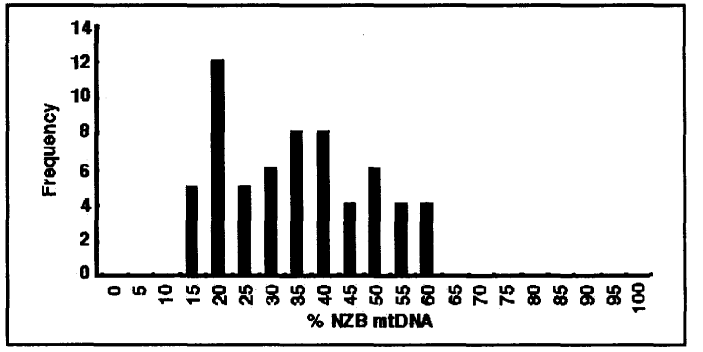
NZB mtDNA Segregation in F2 Mice with Age

3 months

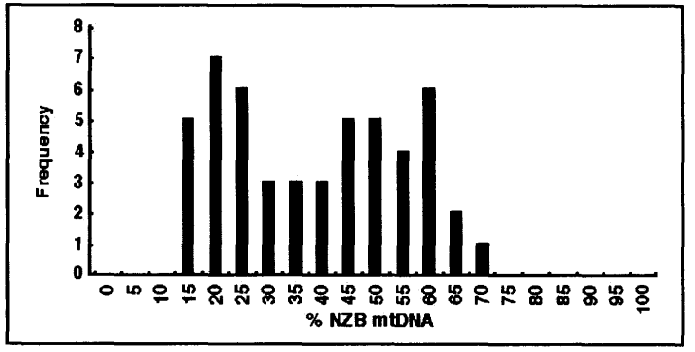
Muscle

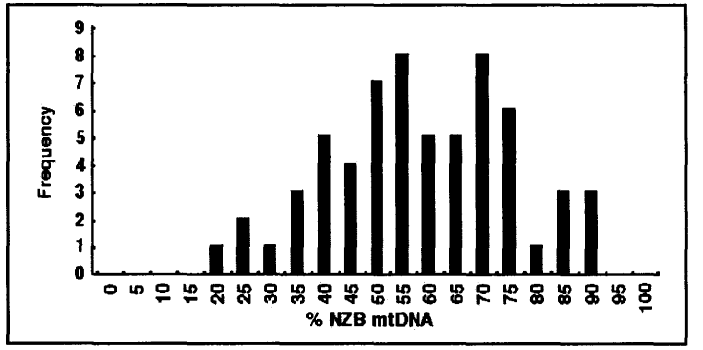
12 months





Kidney



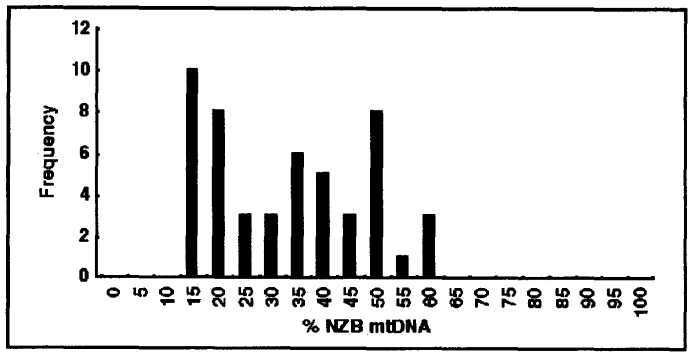


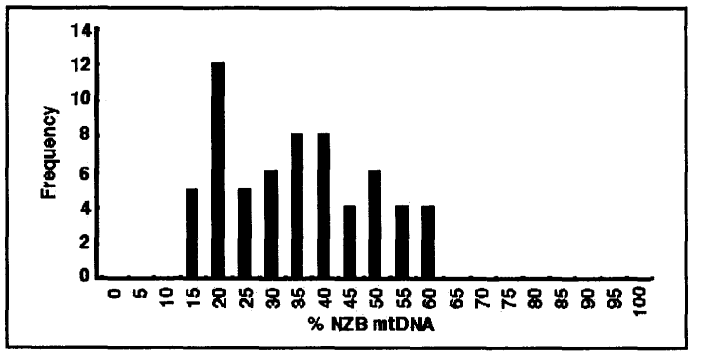
NZB mtDNA Segregation in F2 Mice with Age

3 months

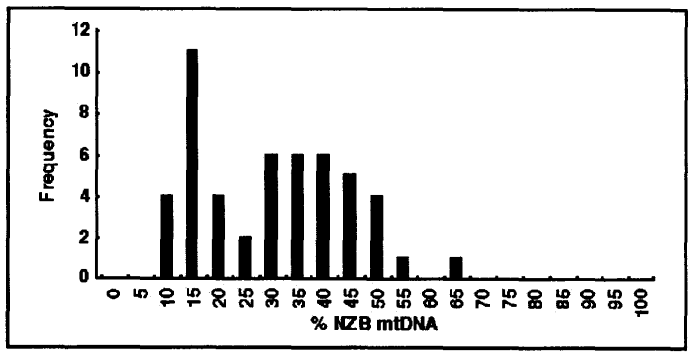
Muscle

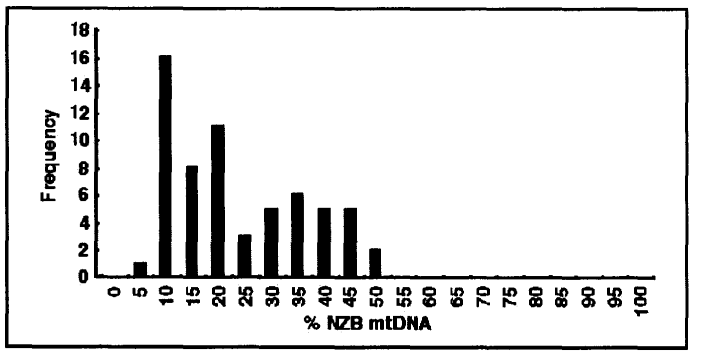
12 months



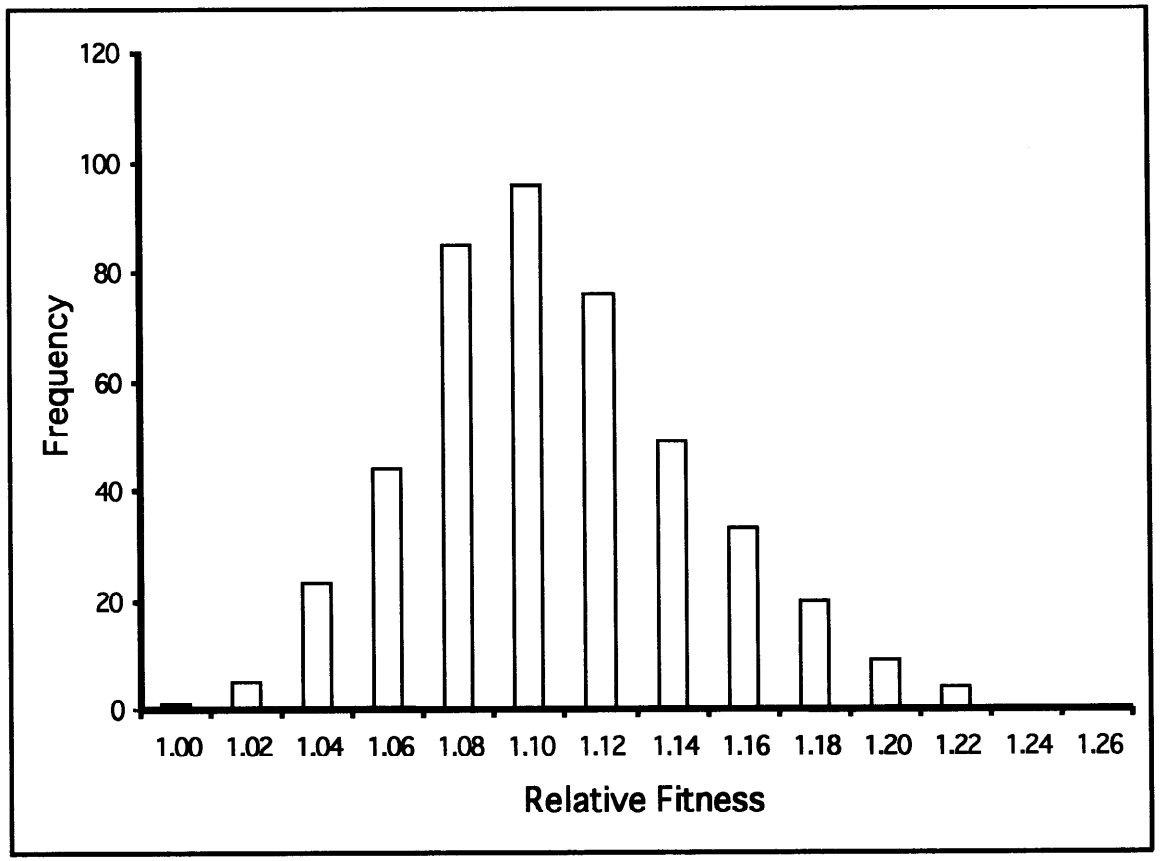


Spleen





mtDNA Selection in the Liver of 3 mth F2 Mice



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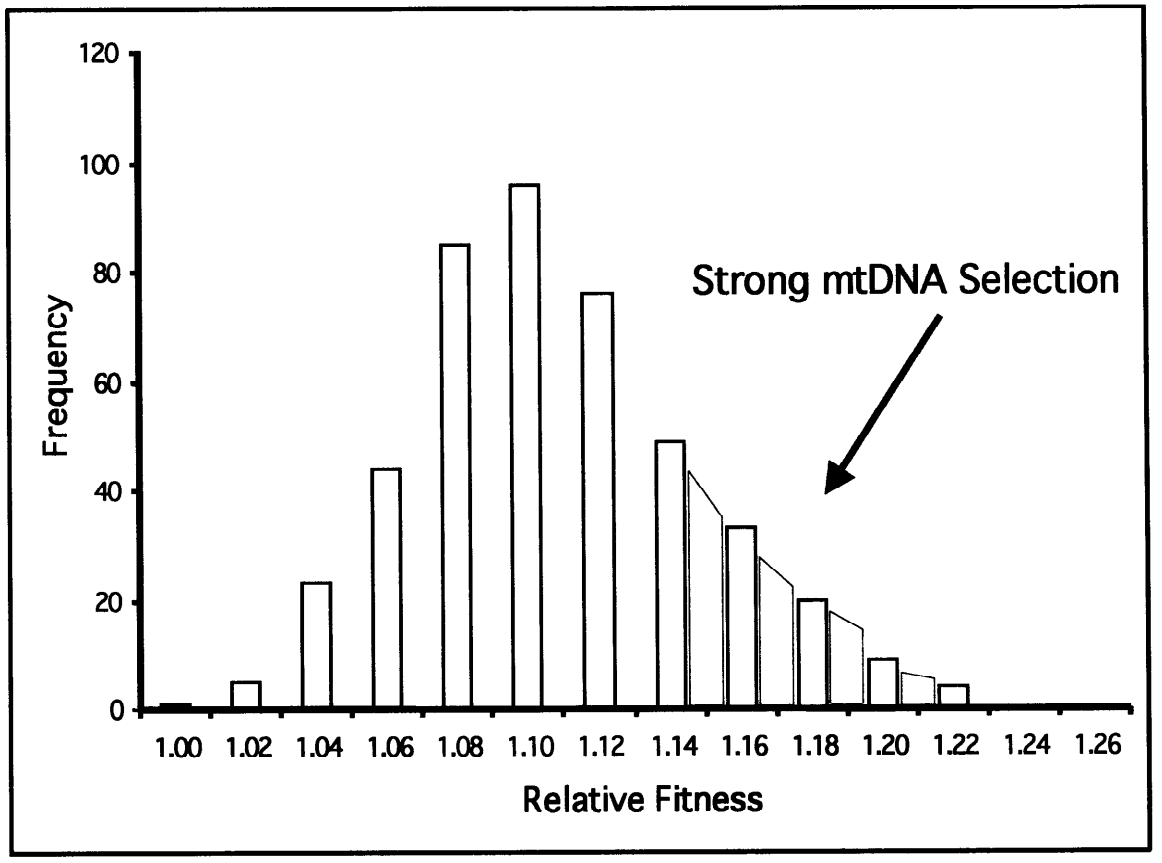
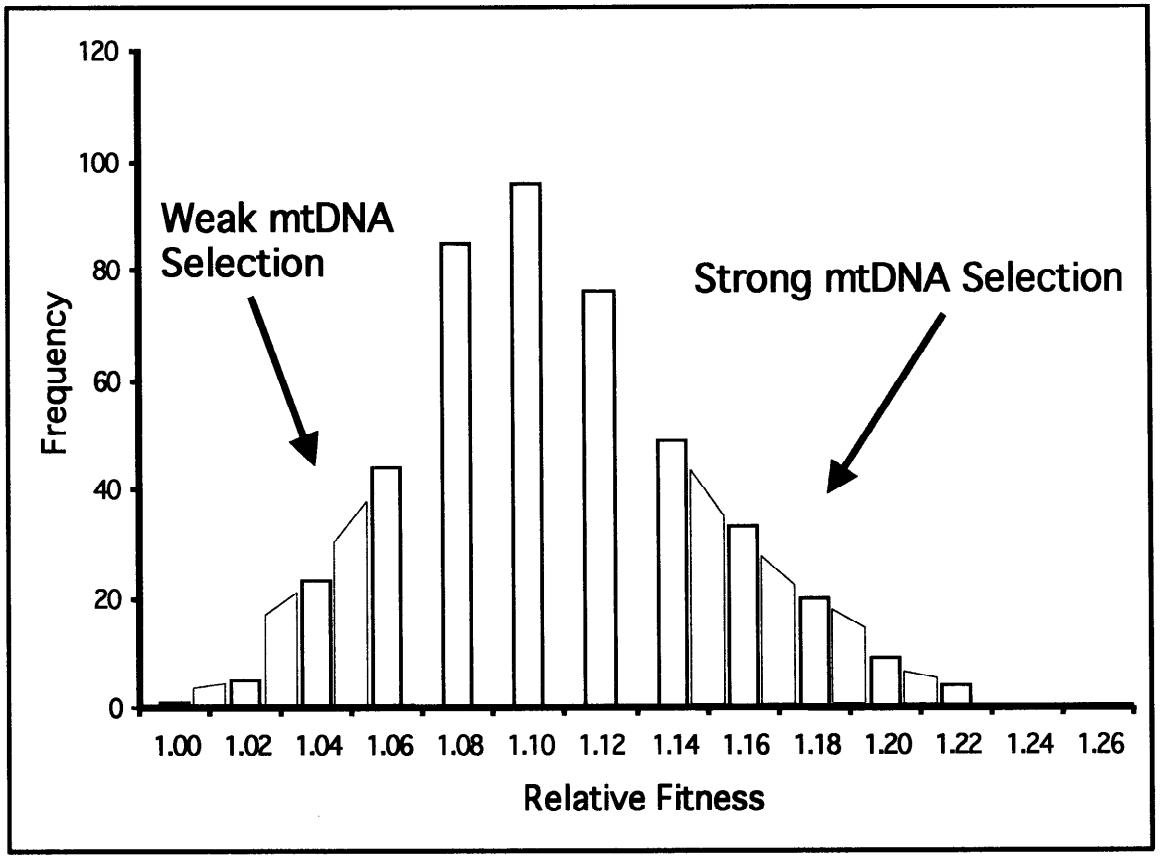


mtDNA Selection in the Liver of 3 mth F2 Mice



Genome Scan

- Test mice at 3 and 12 months
- 50 mice at 3 mths; 60 mice at 12 months
- CA repeat markers spaced ~25 cM
- QTL linkage by interval mapping, MapManager
- High resolution mapping using a dense panel
- Narrow interval in liver using large number of F2 animals

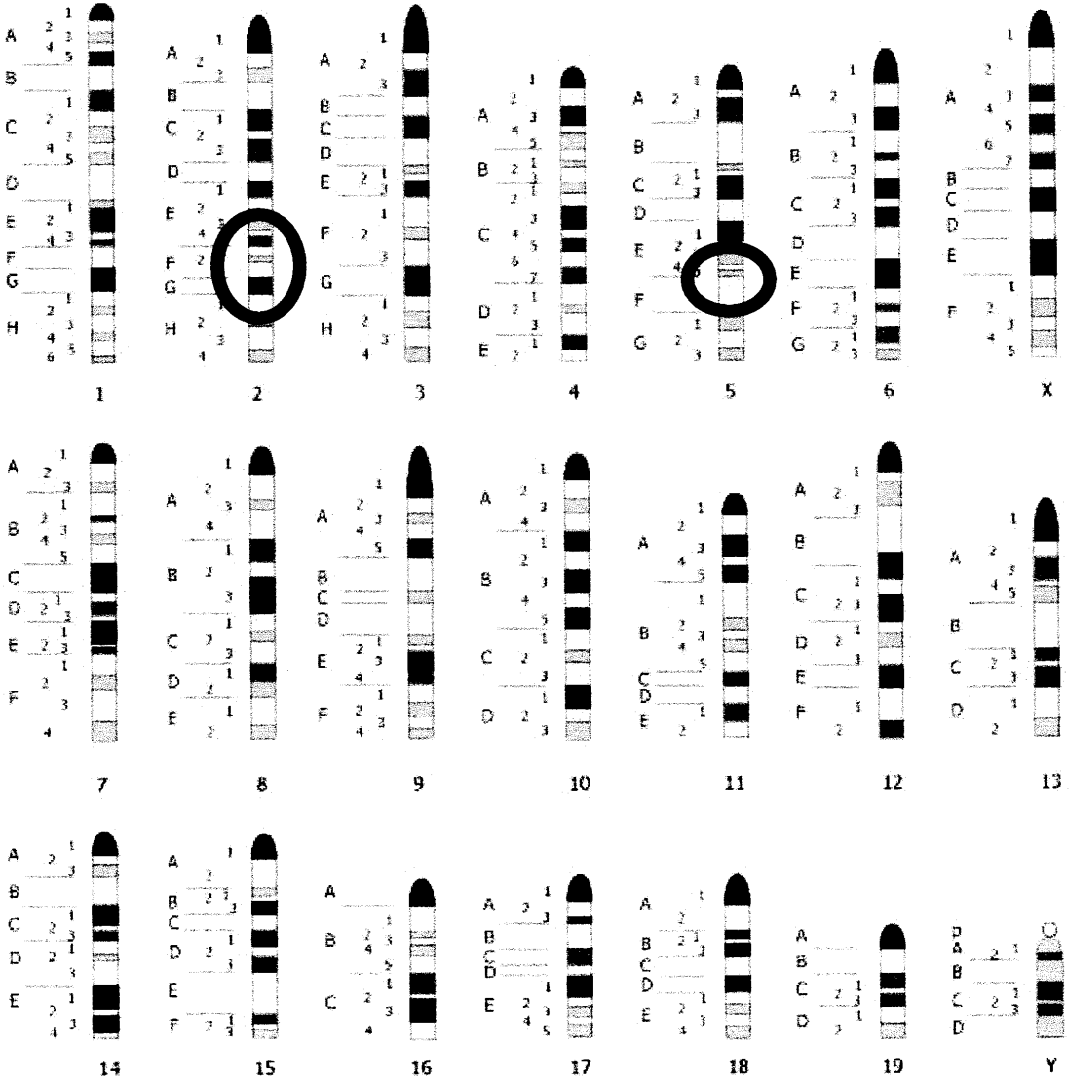
Genome Scan

3 months

Liver - CH 5
LOD 34.4 / 37 %
Dominant

Kidney - CH 2
LOD 4.4 / 17 %
Recessive

Spleen - could not score
phenotype

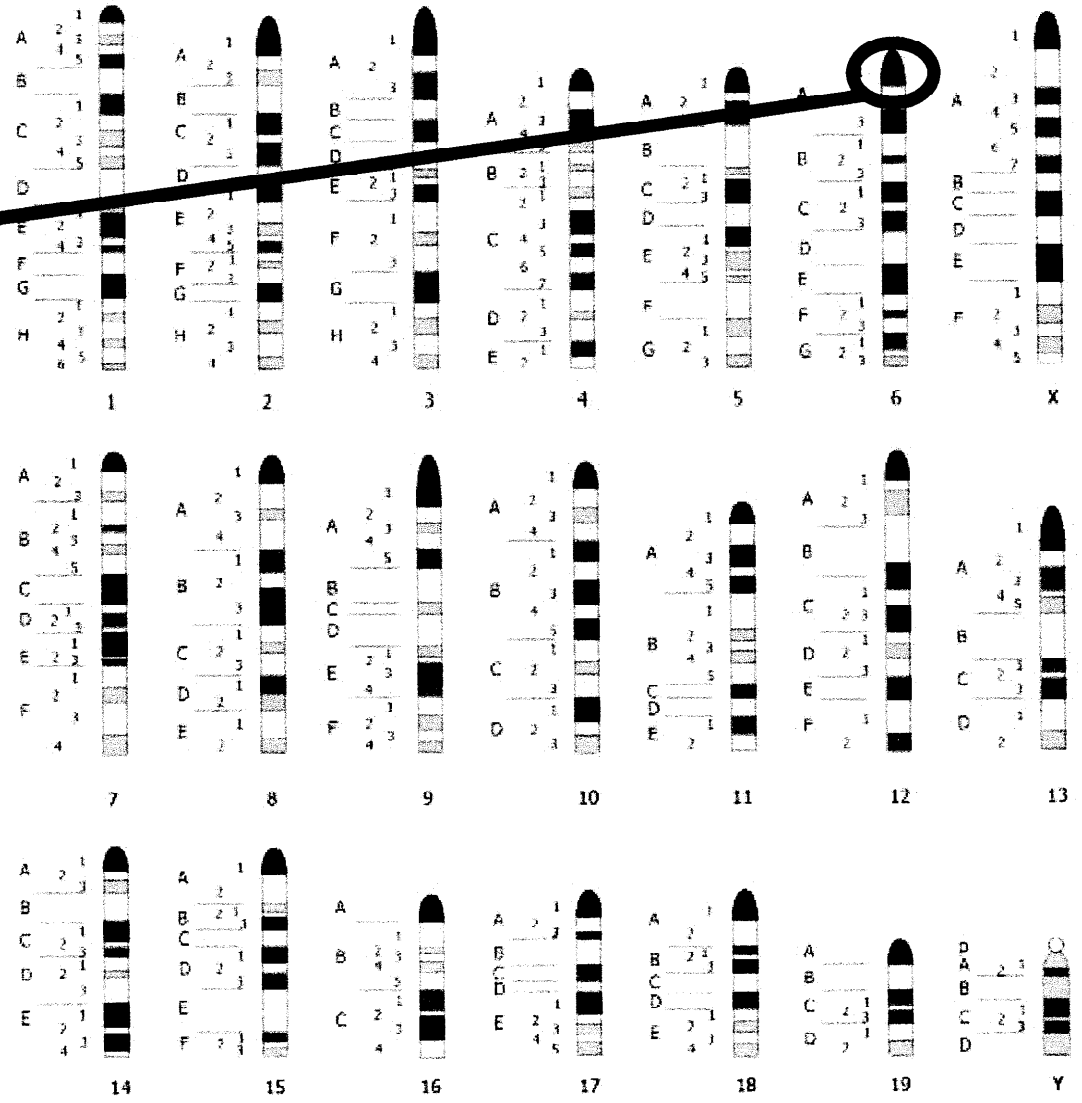


Genome Scan

12 months

Liver - no linkage

Kidney -CH 6
LOD 3.0 / 14 %
Additive



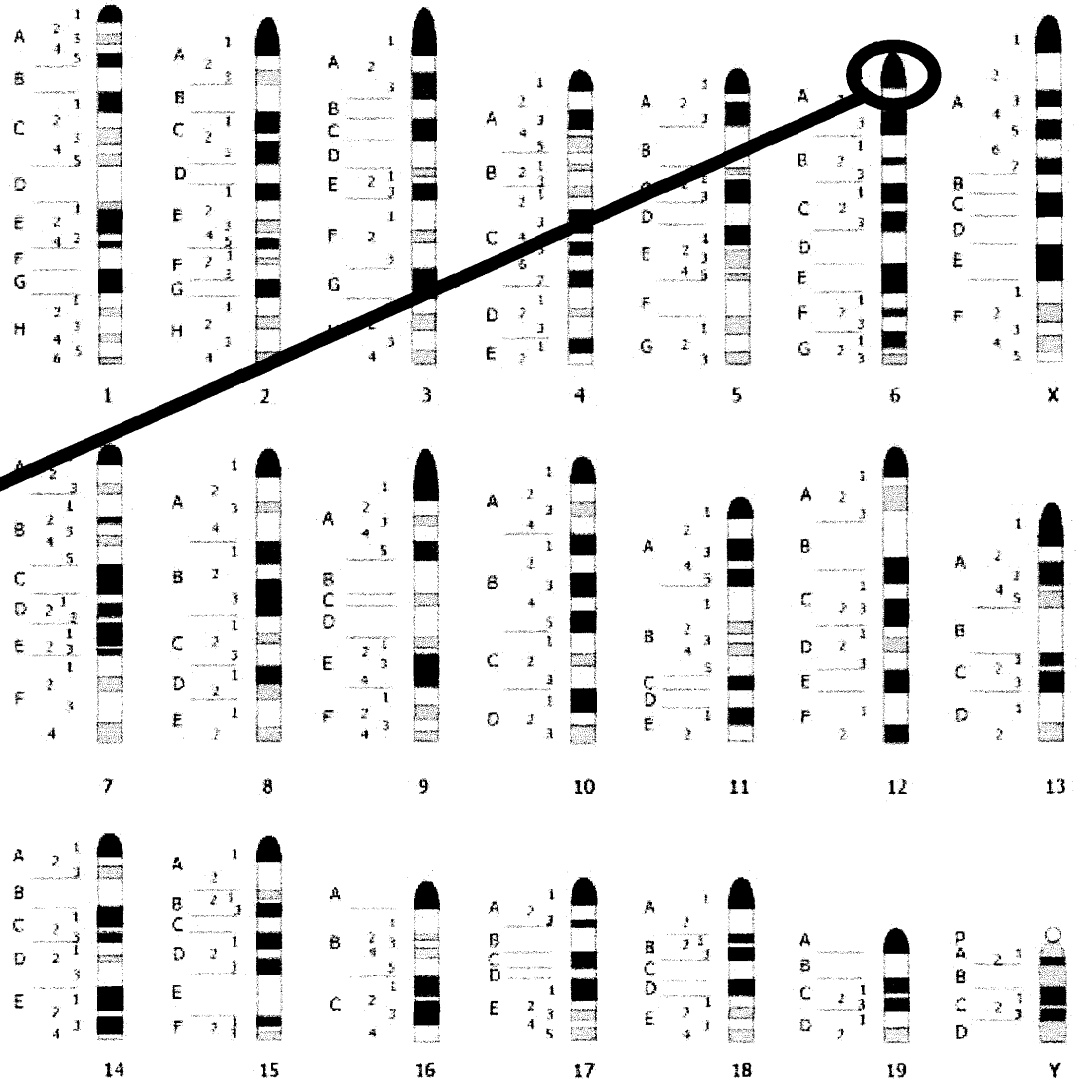
Genome Scan

12 months

Liver - no linkage

Kidney - CH 6
LOD 2.6 / 14 %
Additive

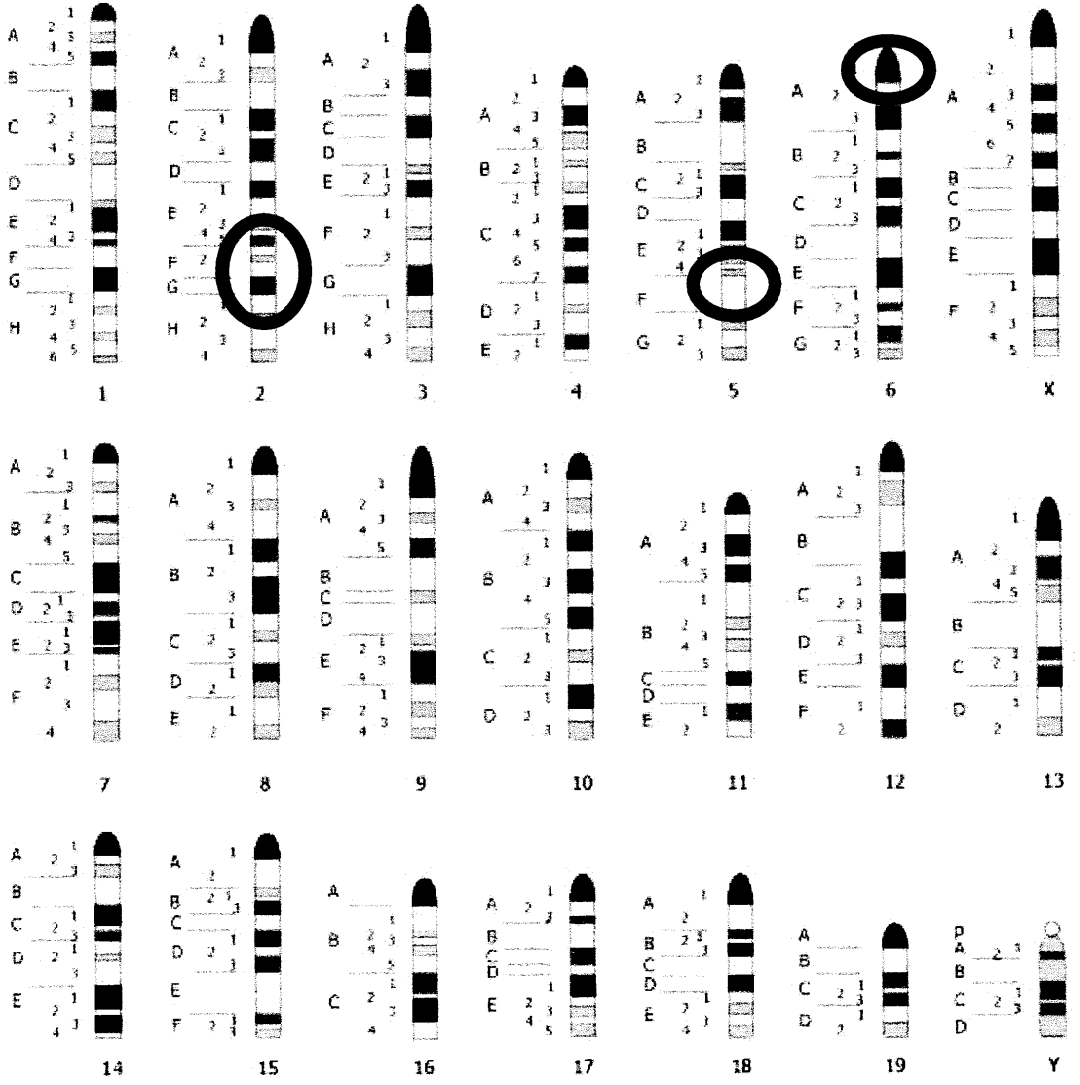
Spleen - CH 6
LOD 4.4 / 19 %
Additive

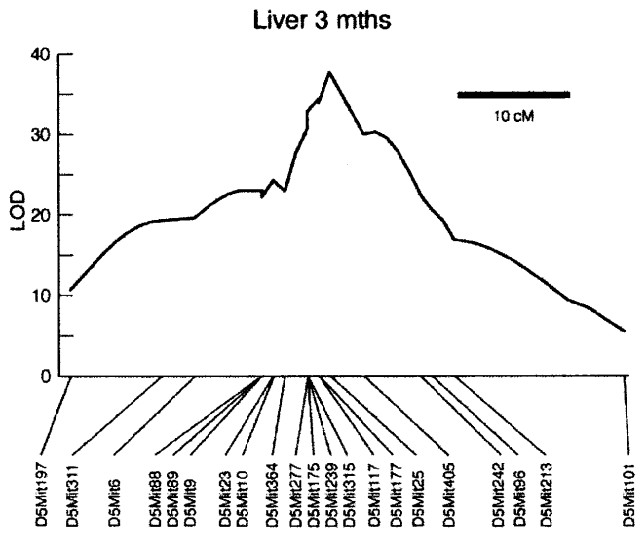


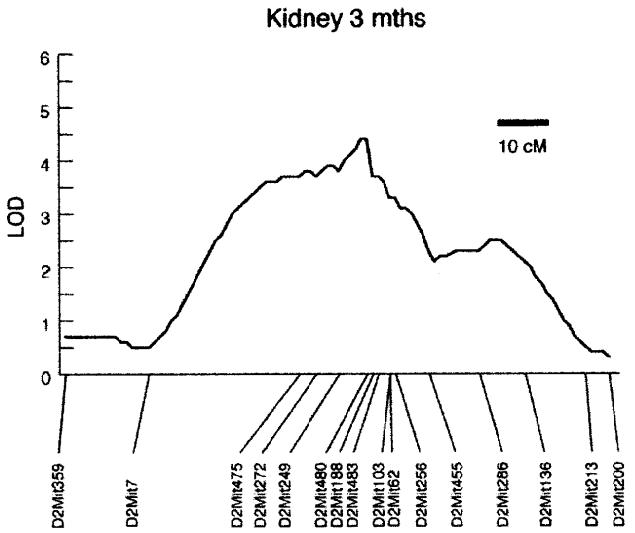
Genome Scan

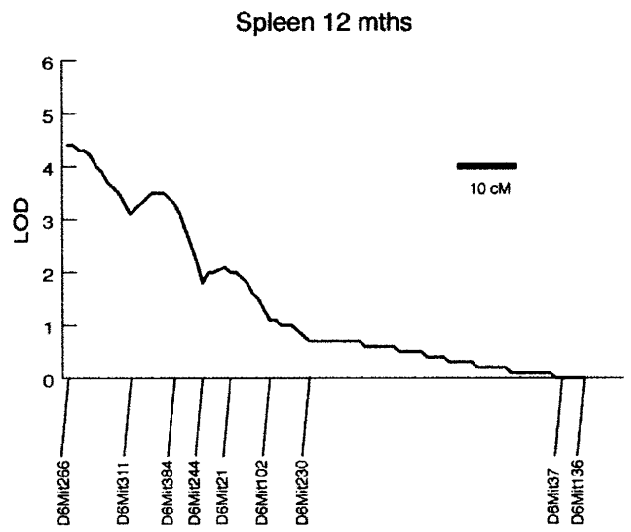
mtDNA selection

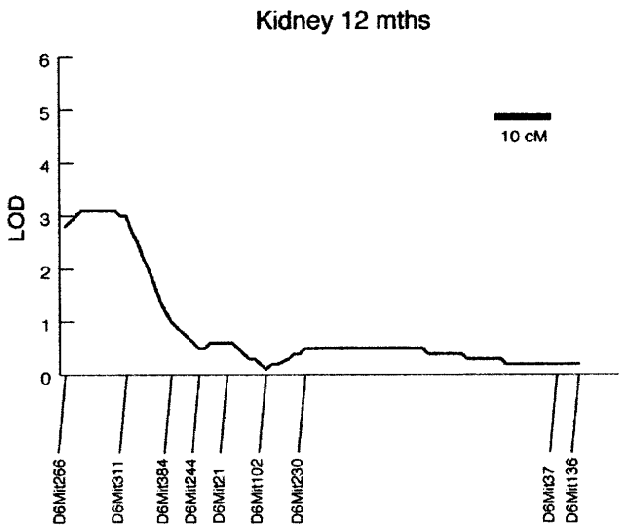
3 loci




Liver 3 mths
LOD
40
30
20
10
0
10 cM
D5Mit197
D5Mit311
D5Mit6
D5Mit88
D5Mit89
D5Mit9
D5Mit23
D5Mit10
D5Mit364
D5Mit277
D5Mit175
D5Mit239
D5Mit315
D5Mit117
D5Mit177
D5Mit25
D5Mit405
D5Mit242
D5Mit96
D5Mit213
D5Mit101


Kidney 3 mths
LOD
6
5
4
3
2
1
0
10 cM
D2Mit359
D2Mit7
D2Mit475
D2Mit272
D2Mit249
D2Mit480
D2Mit188
D2Mit483
D2Mit103
D2Mit62
D2Mit256
D2Mit455
D2Mit286
D2Mit136
D2Mit213
D2Mit200


Spleen 12 mths
LOD
6
5
4
3
2
1
0
10 cM
D6Mit266
D6Mit311
D6Mit384
D6Mit244
D6Mit21
D6Mit102
D6Mit230
D6Mit37
D6Mit136


Kidney 12 mths
LOD
6
5
4
3
2
1
0
10 cM
D6Mit266
D6Mit311
D6Mit384
D6Mit244
D6Mit21
D6Mit102
D6Mit230
D6Mit37
D6Mit136

Summary of dsmd loci

Tissue	Month	Chromosome / Locus	Designation	LOD	%	Level of Significance	Model	mtDNA genotype selection
Liver	3	5 / D5Mit25	dsmd-1	34.4	37	P=0.0	dominant	NZB
Kidney	3	2 / D2Mit480	dsmd-2	4.4	17	P=0.0015	recessive	NZB
	12	6 / D6Mit266	dsmd-3	3.0	14	P=0.008	additive	NZB
Spleen	12	6 / D6Mit266	dsmd-3	4.4	19	P=0.0005	additive	BALB

Conclusions

- Transmission of mtDNA sequence variants is primarily stochastic
- Tissue-specific nuclear genetic control of mtDNA segregation
- Structure of the mtDNA nucleoid ?

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