Comparison of biological features of major rat strains utilizing for 2-year carcinogenicity

Item		**Wistar-Hannover GALAS**	**Sprague-Dawley IGS**	**Fischer F344**
		Closed colony	Closed colony	Inbred
Survival rate	1 year	90-100%	80%	90%
	2 year	70-80%	30-40% (40-60% low protein diet)	50-60%
Body weight (2 year)	Male	0.6 – 0.7 kg (Max 0.8)	0.7 – 1.3 kg (Max 1.6)	0.6 – 0.7 kg (Max 0.8)
	Female	0.3 – 0.4 kg (Max 0.6)	0.5 – 0.8 kg (Max 1.4)	0.3 – 0.4 kg (Max 0.6)
Hematology		Not special	Not special	Not special
Biochemistry		Not special	Not special	Not special
Urinalysis		Higher incidence Occult blood	Sporadically Occult blood	Not special
Nephropathy		Not very high After 26 weeks	High incidence After 13-15 weeks	Not very high After 26 weeks
Other diseases (disadvantages)		Spontaneous Thyroid gland dysplasia* (approx. 10%)	Higher incidence of mineralisation, vascular lesions and liver disorders	Testicular tumors (100%)

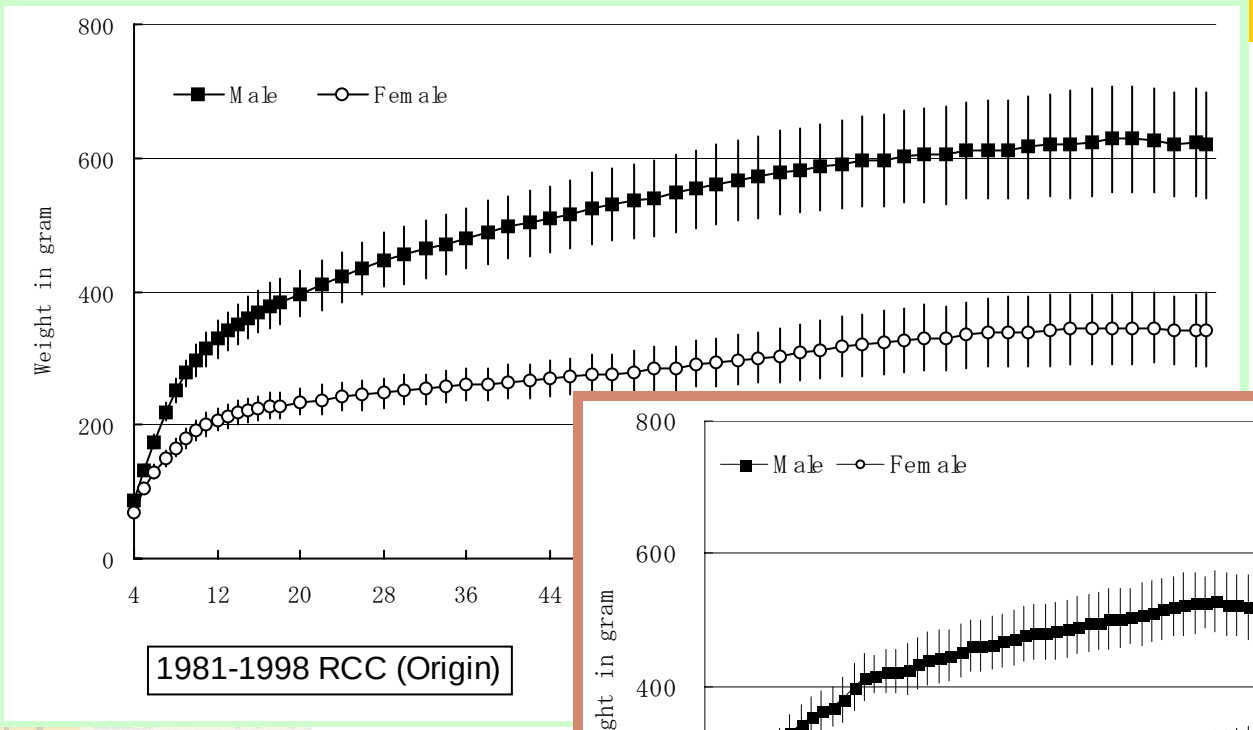
* Thyroid gland dysplasia could be possible congenital disorder related with hereditary defects
No abnormality in phenotype and biochemistry (including T3, T4, TSH).
No effects on neoplasia development in 2-year carcinogenicity

Comparison of biological features of major rat strains utilizing for 2-year carcinogenicity

Item		Wistar-Hannover GALAS	Sprague-Dawley IGS	Fischer F344
Spontaneous tumors				
Systemic neoplasia		<5% including thymic lymphoma	5 % Higher histiocytic tumor	Up to 70% mailnly myeloic leucemia
Mammary gland tumor		15-20%	70%	15-20%
Pituitary tumor		15% (male) up to 45% (female)	15% (male) 30-50% (female)	10% (male) 35-40% (female)
Testicular tumor		<5%	<5%	100%
General tumor		Not very high	Higher incidence Often rare tumor	Not very high
Market share#	U.S.	20%	70%	standard in NTP
	Europe	80%	10%	rarely used
	Japan	10%	80%	rarely used
Suppliers		Taconic, CLEA	Charles River	Charles River and others.
Historical control data		Not enough recently	Enough Wide variation among breeders	Enough (only NTP) Tendency to BW↑ , Survival↓
Global supply system		**Yes**	Yes?	No
Genetic monitoring system		**Yes**	No	No

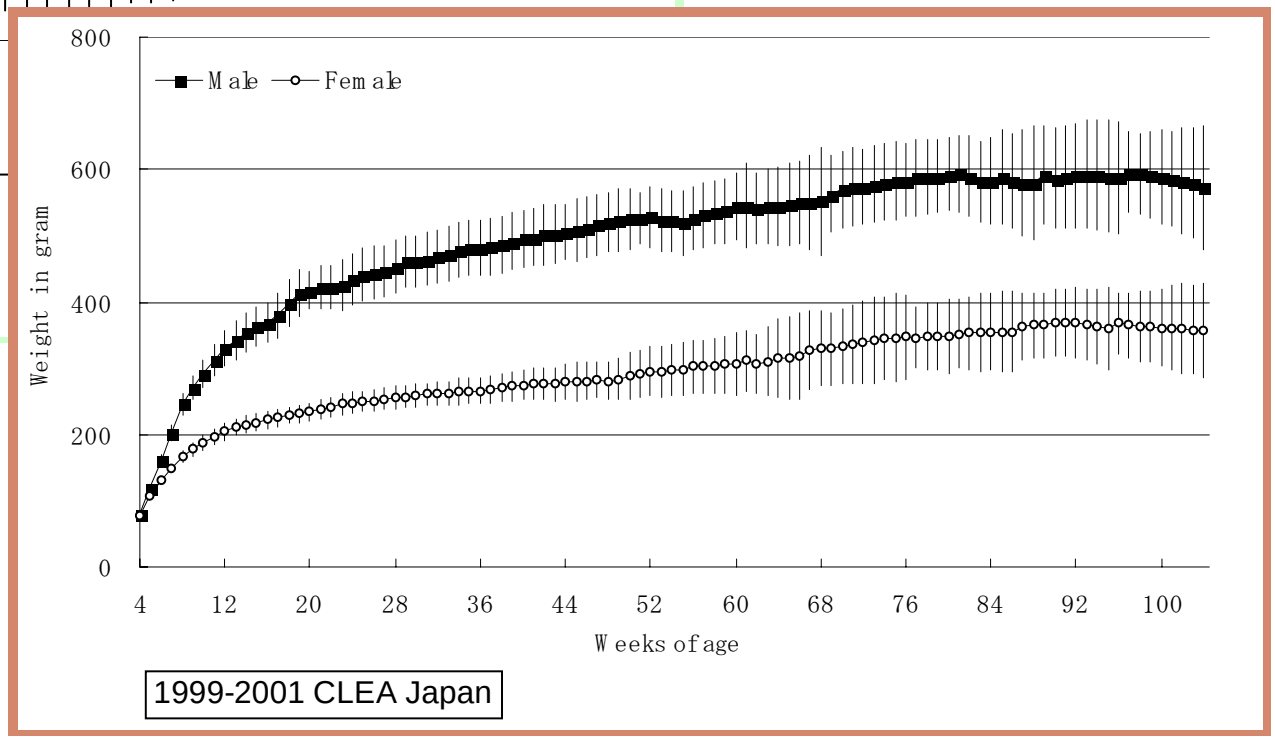
Other promising rat strains include Wistar-Hannover IGS, Wistar-Hannover RCC and traditional Sprague-Dawley.

No change in Body weight curve for 2-year



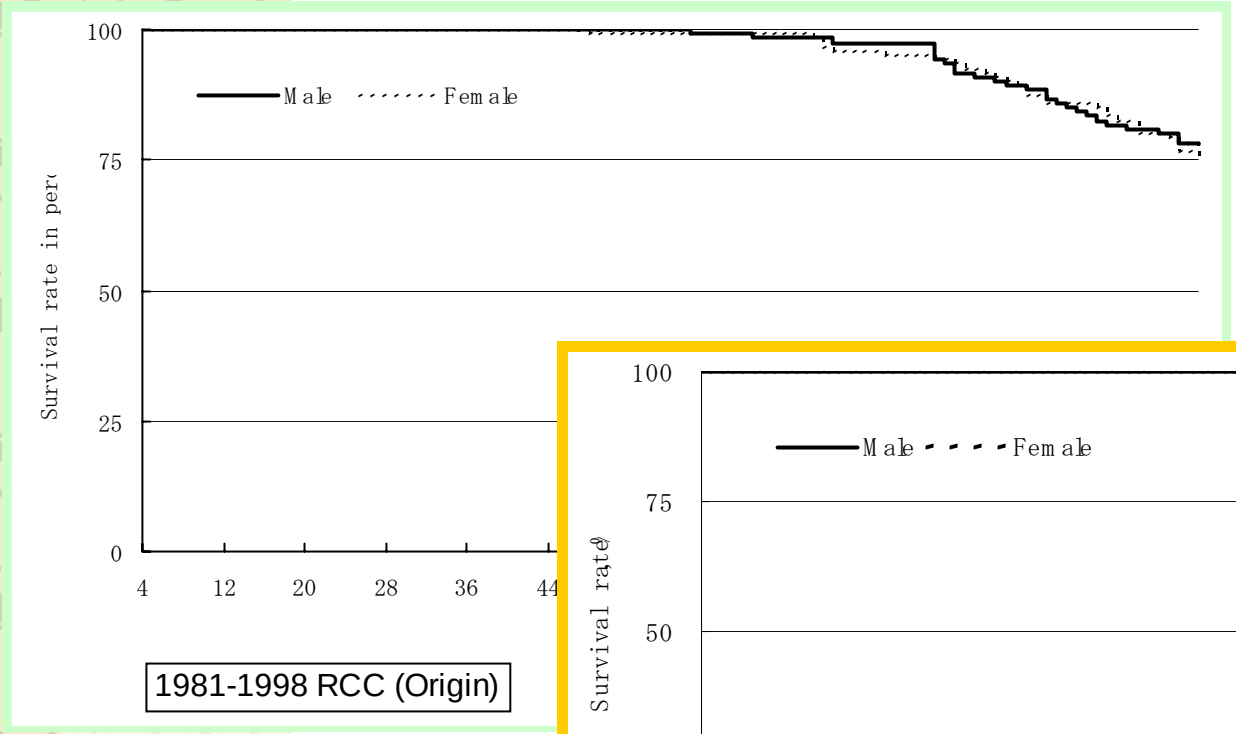
1981-1998 RCC (Origin)

GALAS rats



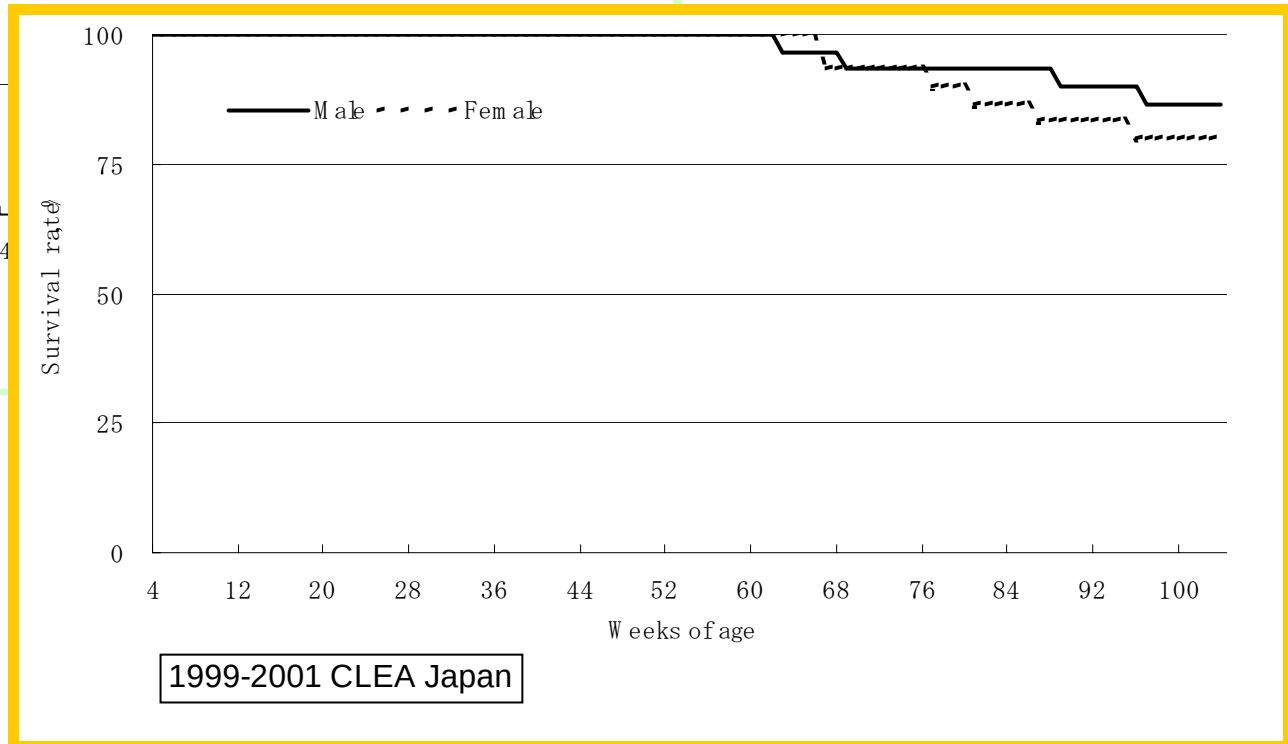
1999-2001 CLEA Japan

No change in Survival rate for 2-year



1981-1998 RCC (Origin)

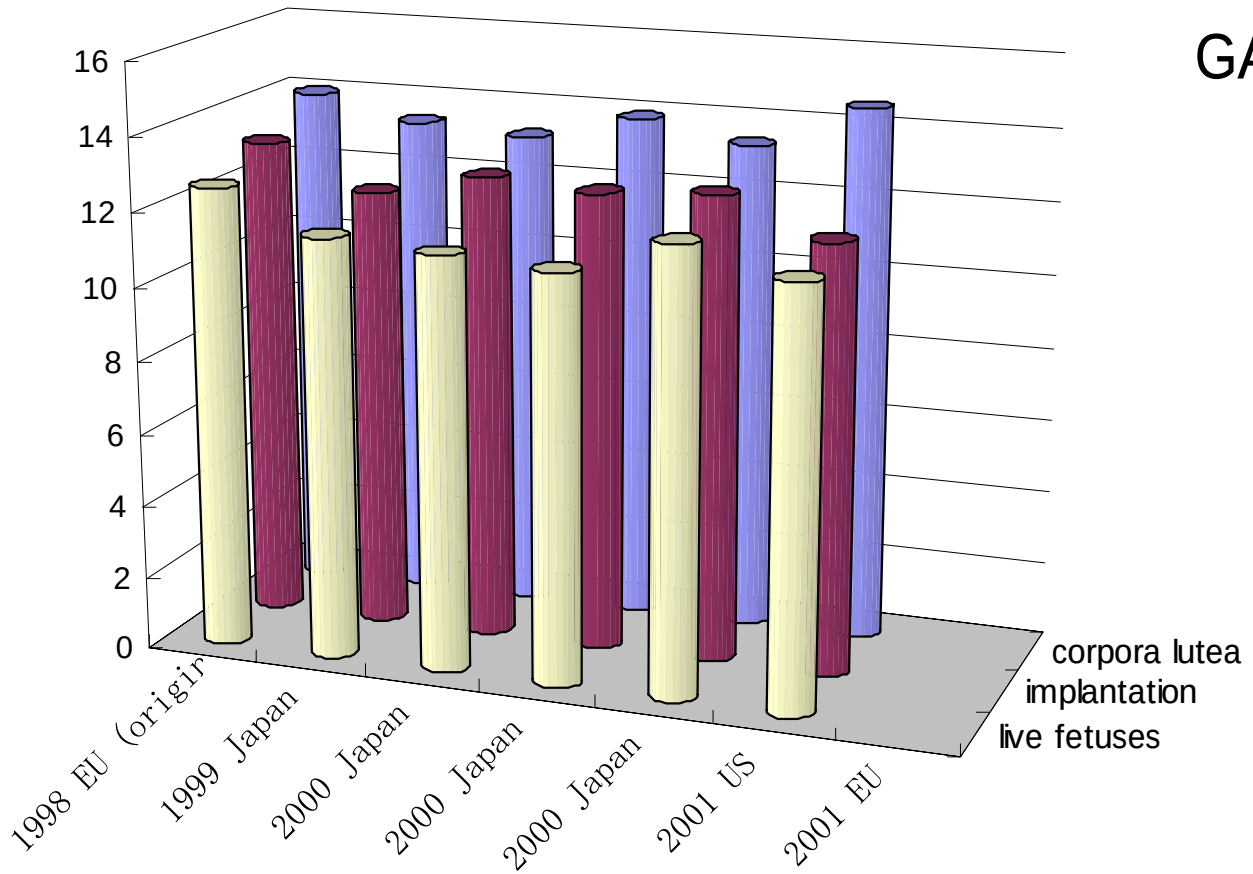
GALAS rats



1999-2001 CLEA Japan

No significant difference on Reproductive
Multi breeding sites and trans-generations

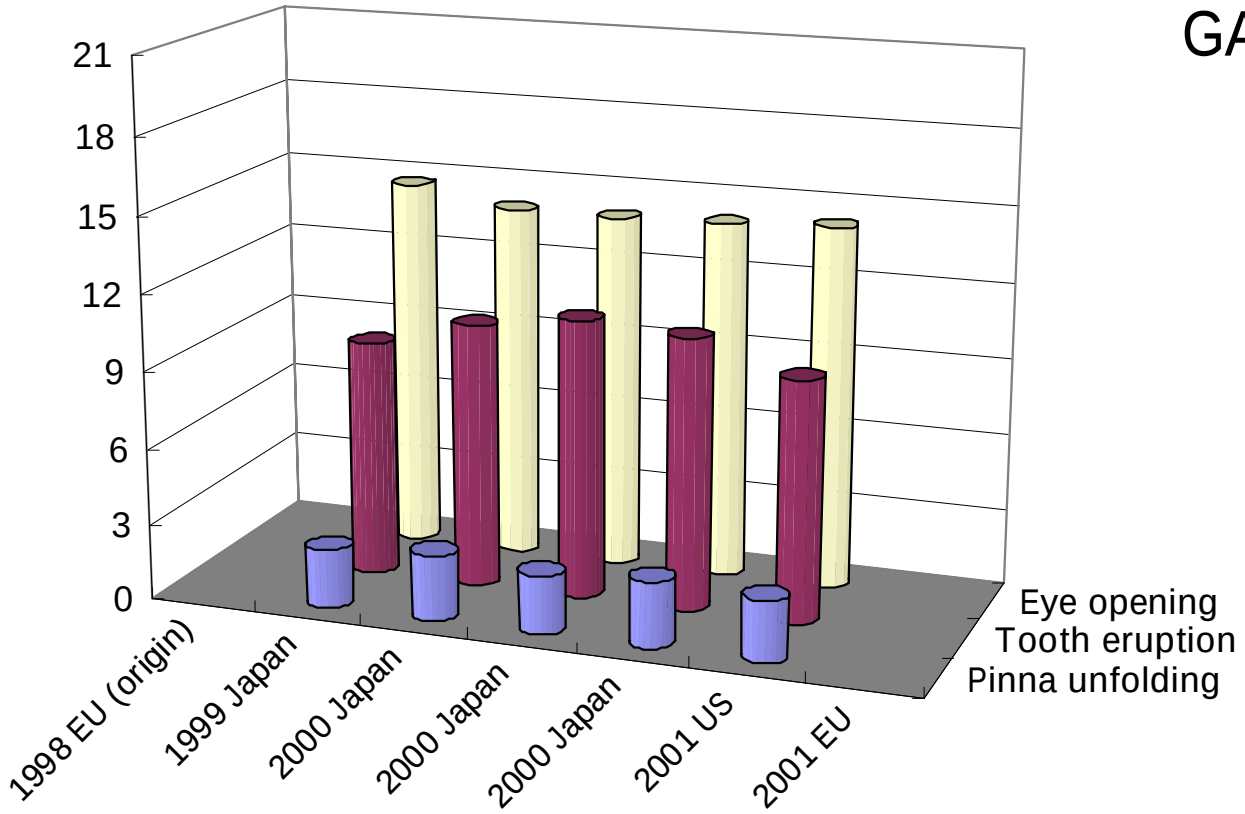
GALAS rats



Need for consecutive data collection in each breeding site

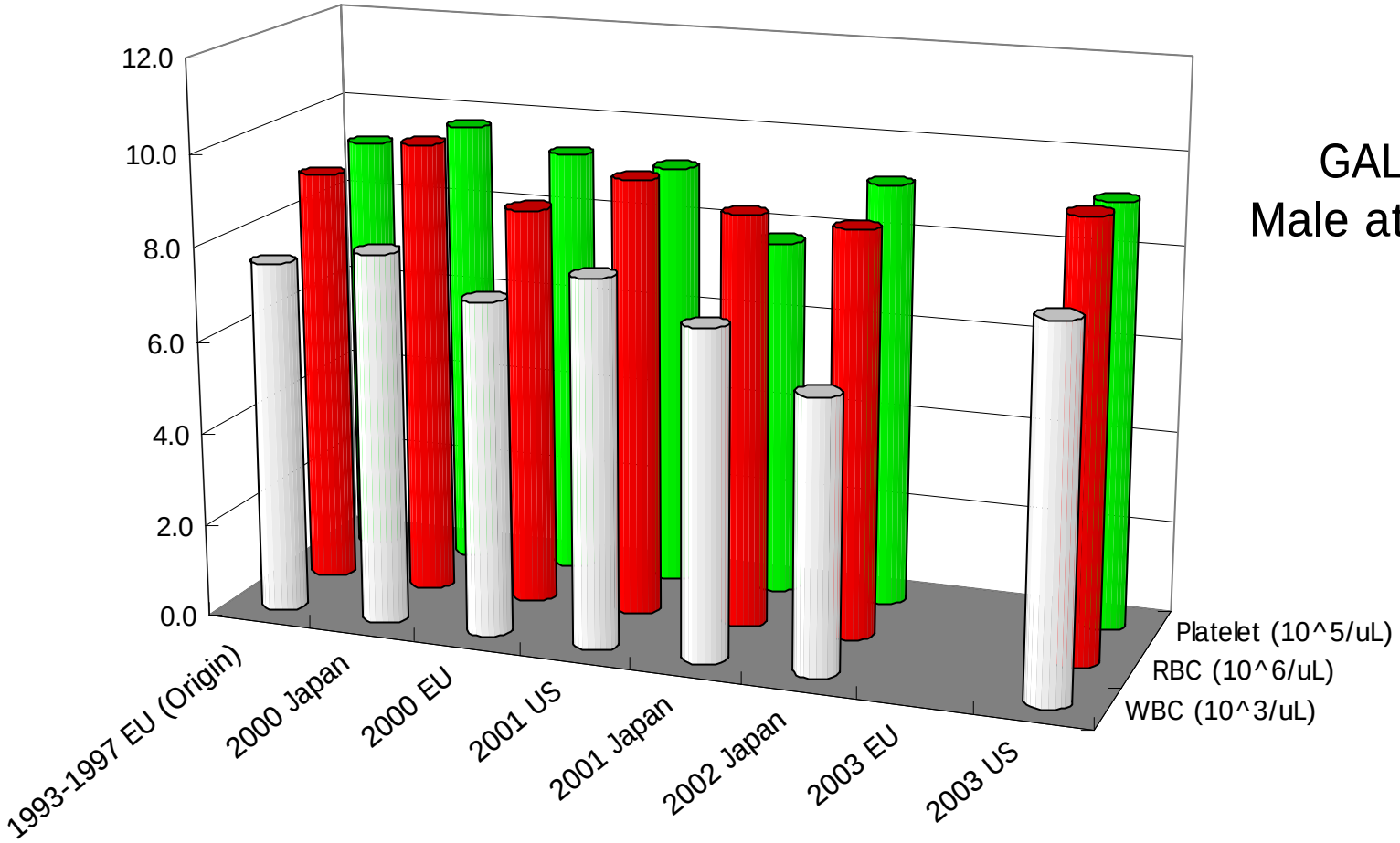
No significant difference on Postnatal Development

Multi breeding sites and trans-generations



GALAS rats
Male

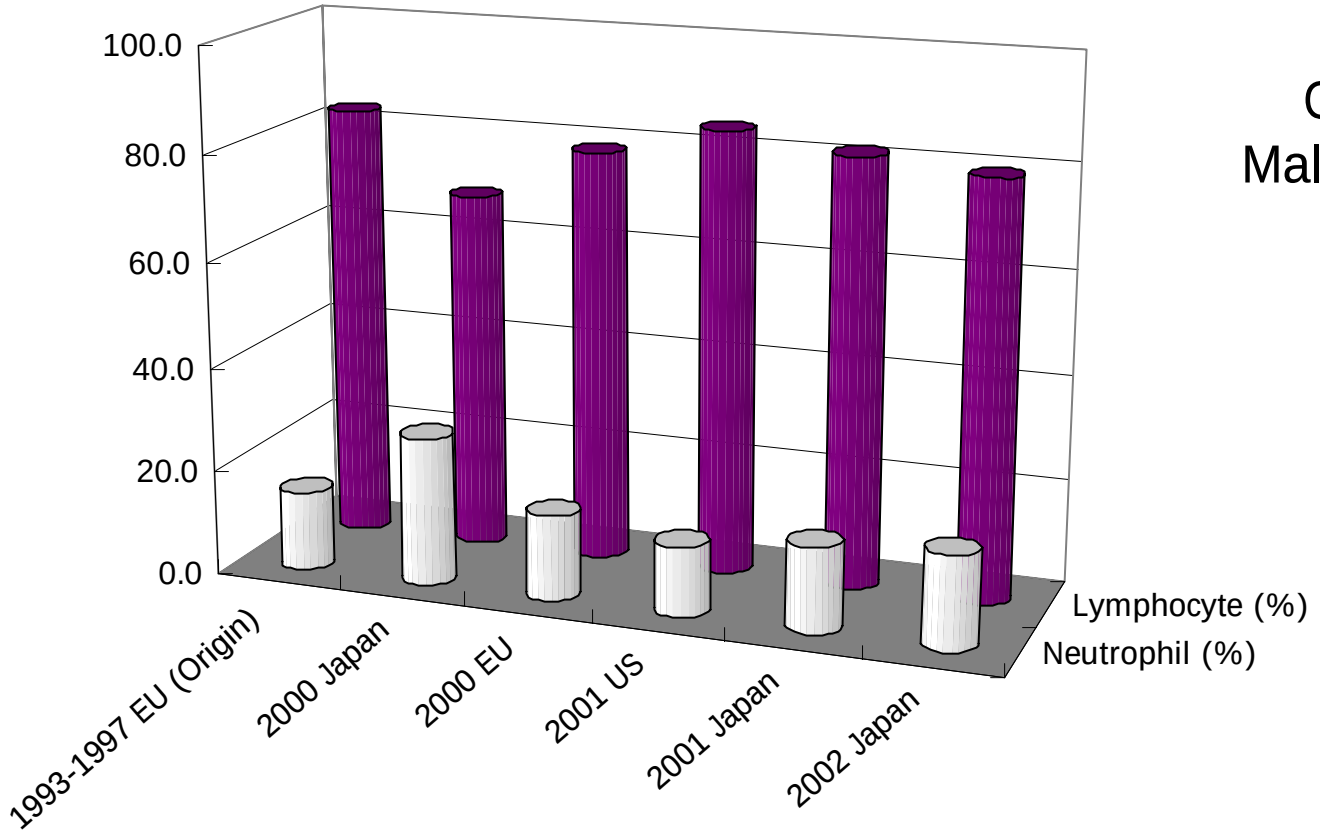
No significant difference on Hematology
Multi breeding sites and trans-generations



GALAS rats
Male at week 18*

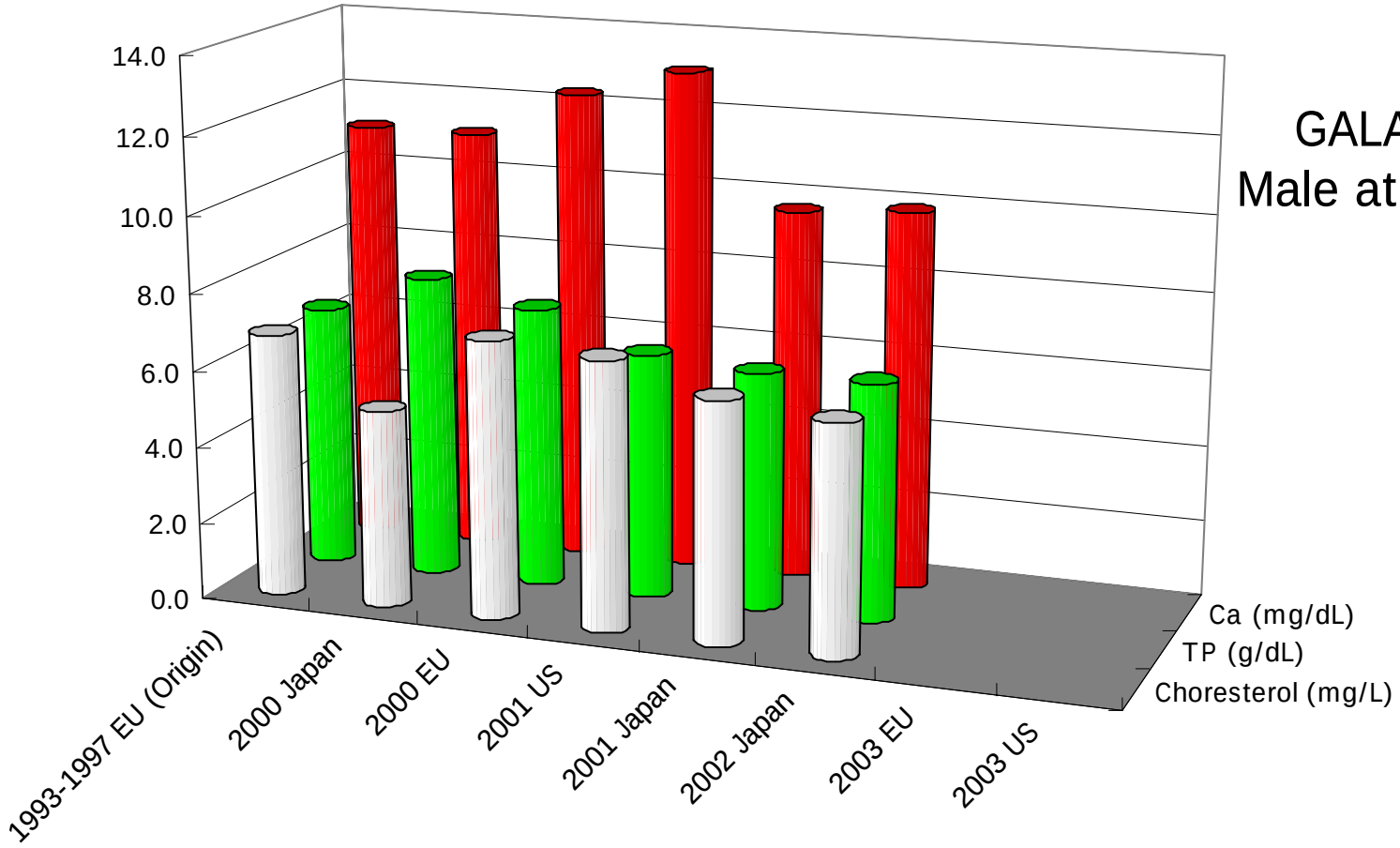
* 3-month dose-finding study for 2-year carcinogenicity

No significant difference on Hemogram
Multi breeding sites and trans-generations



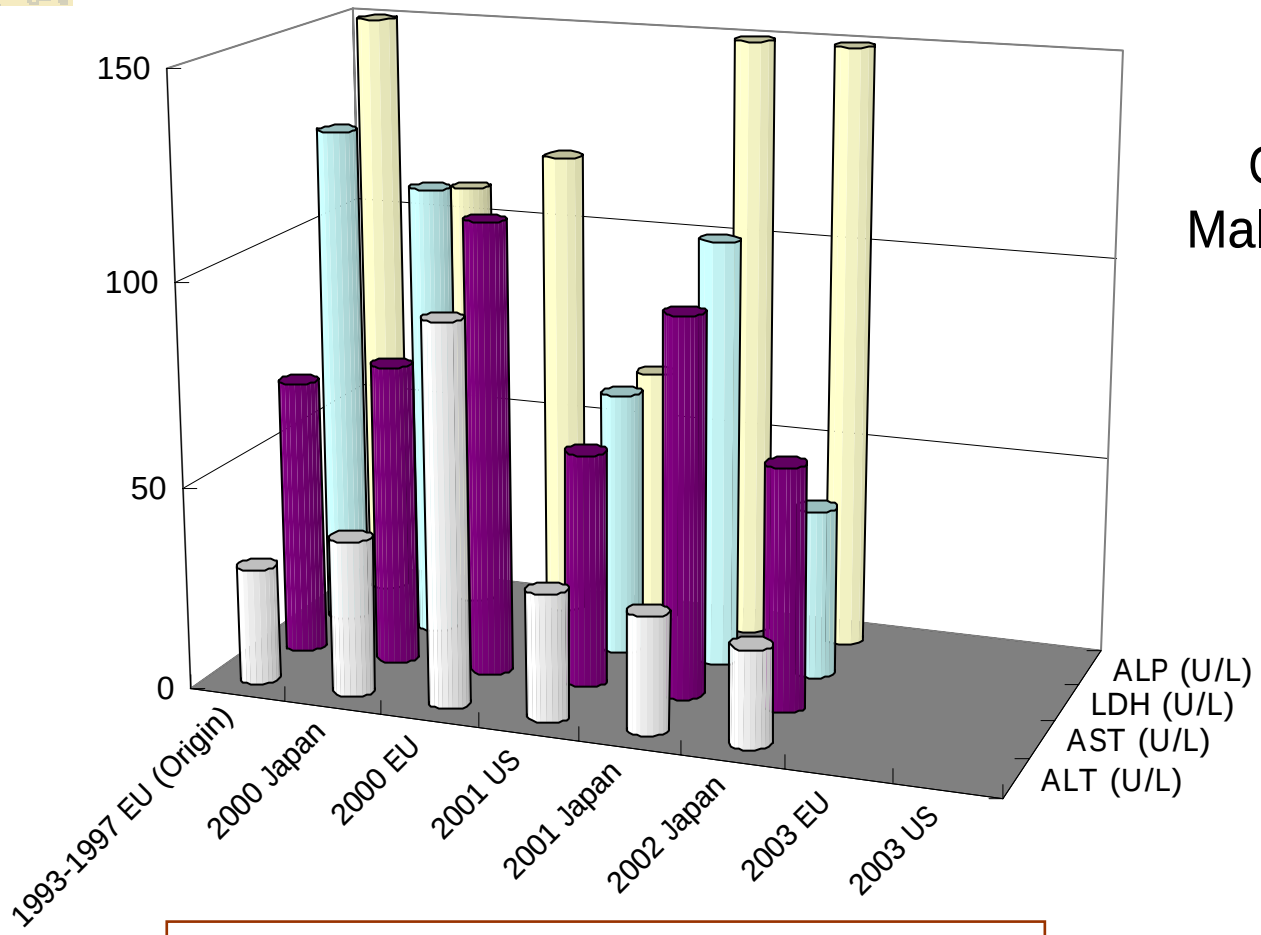
GALAS rats
Male at week 18

No significant difference on Biochemistry
Multi breeding sites and trans-generations



GALAS rats
Male at week 18

Unlikely comparison on enzyme activities



GALAS rats
Male at week 18

Need for uniformed assay method !!

Necessity of Global standardized closed colony rats

- Issues of current closed colony rat strains
  - Animals, with the same strain name, are not always identical
  - Genetic drifting by generation & by breeding site, with no major impact on phenotype profile
  - Insufficient information available on genetic profile
- Need adequate breeding system to avoid genetic drifting
  - Embryo preservation for risk management
  - Harmonized minimum health profile
  - Quality network between breeding sites (global supply system)
- Need precise genetic monitoring system based on specifications
  - Only way to assure the reproducibility and comparability of animal study
  - Data-oriented quality assessment

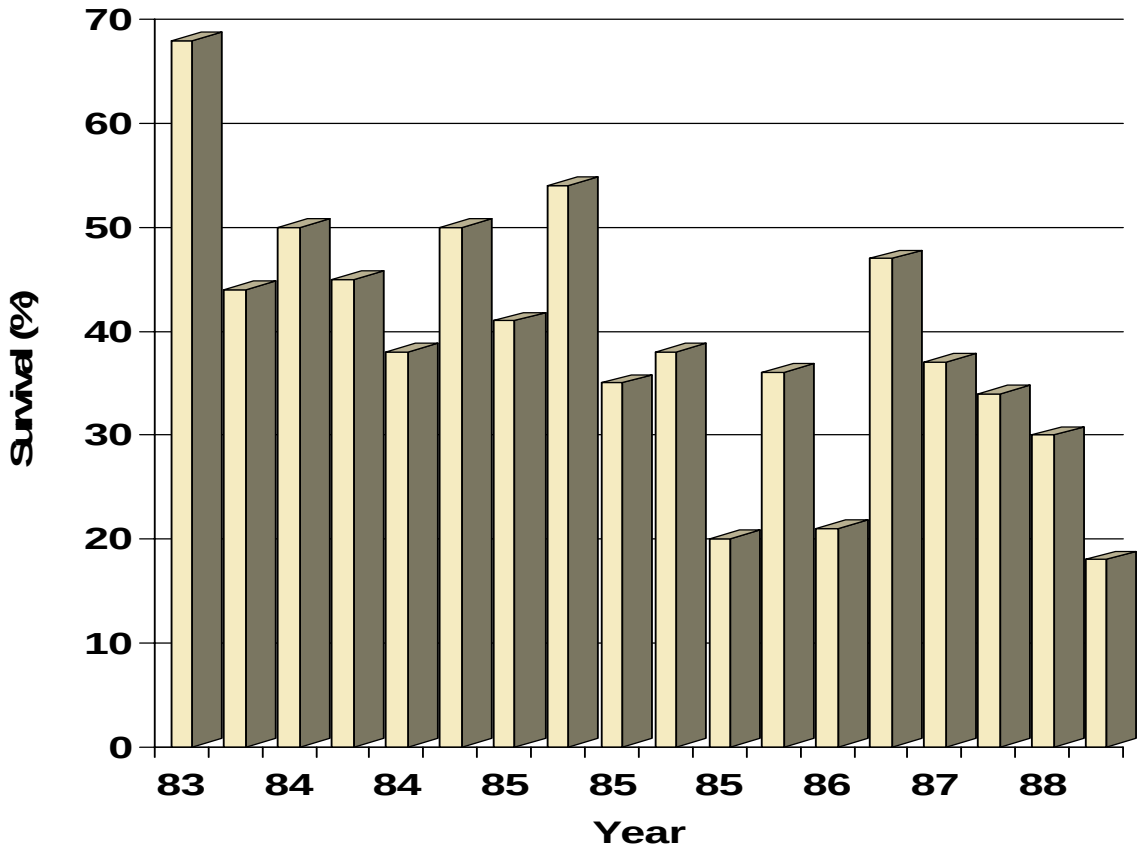
Concept of Global standardized closed colony rats

- Selection of most relevant closed colony rats strain
  - Sufficient background data (in this case for 2-year carcinogenicity study)
  - Unlikely spontaneous lesions affecting study evaluation

- Establishment of
  - Genetic monitoring system (Not Phenotype)
    - Specifications of genetic profiles and genetic polymorphisms
    - Trans-generational stability of gene frequencies
  - Reliable production method (maintained rotation system)
    - No artificial selection other than reproductivity
    - Global supply system between breeders in US, EU and Japan

Trend towards reduced survival

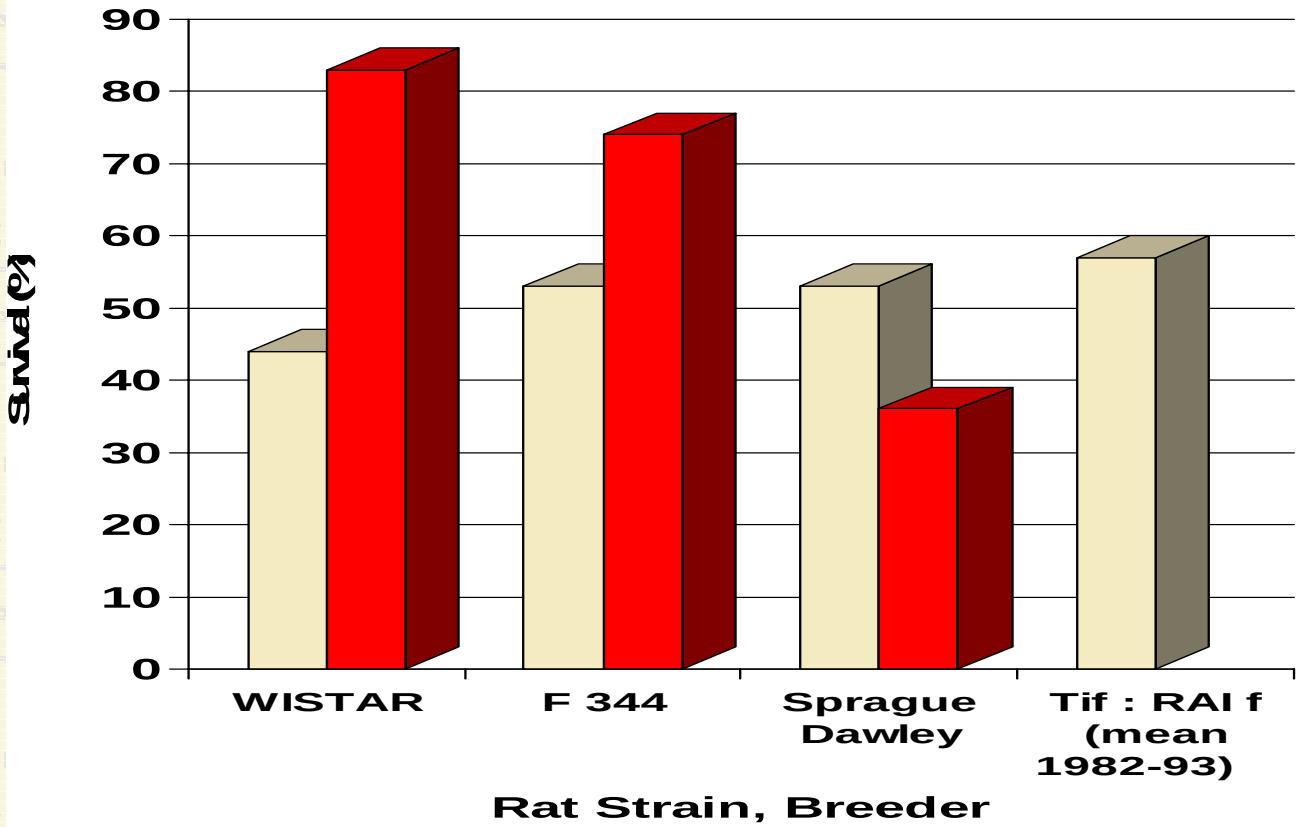
Sprague Dawley Rats (male) at Week 104



Dr. Van Cauteren et al. the Japanese Society of Laboratory Animal Science, 2003

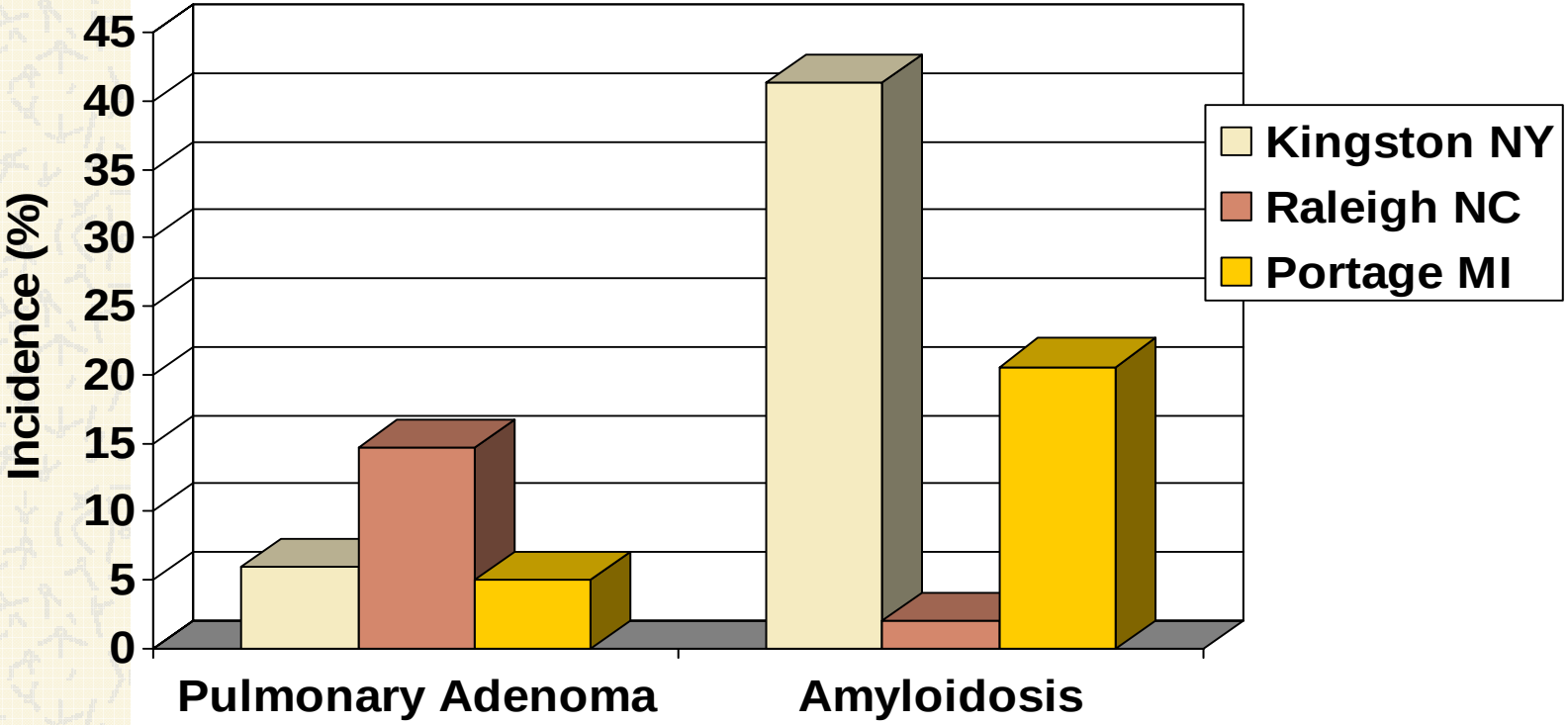
Longevity of rats by strain and breeder

Survival of Male Rats at Week 104



Dr. Van Cauteren et al. the Japanese Society of Laboratory Animal Science, 2003

Incidence of pulmonary adenoma and amyloidosis in CD-1 mice from 3 different sites of the same breeder



Dr. Van Cauteren et al. the Japanese Society of Laboratory Animal Science, 2003

Example : Drug ABC

Proposed Carcinogenicity Study to FDA

Carcinogenicity Study Proposal

- **Sprague-Dawley rat**, 2 years
- US CRO
- Dietary administration: 0, 0, 5 15, 45 mg/kg
- Based on MTD - from 3 month toxicity study - dose limiting hepatic and CV effects (CV known for class to increase in severity)

CAC Response

- **No concurrence**
  - Toxicity profile and sensitivity changed with changing study sites
  - No *hepatic* effects in EU-based study; No *renal* effect in Japan-based study,
  - CV effects not exposure or duration of treatment related across studies
  - Exposure by dietary administration variable by study site
  - No dose ranging at US facility
- Response may be influenced by animal source, husbandry, or diet

- **CONSEQUENCE**
  - **1 year delay in conduct of Carcinogenicity Study to repeat DR in US CRO**

Genetic monitoring in closed colony animals

CIEA/CLEA *GALAS rat research unit*

Experimental Animals

- Inbred

  maintained by sib mating to make a genetically homogenous population

  all animals show same genotype

- non-inbred,Outbred or Closed colony

  maintained by random mating to conserve a genetic heterogeneity

  all animals show different genotype

How should we do their genetic monitoring?

Genetic monitoring in closed colony animals

Traditional method

- Genotype a limited number of samples from a colony
  estimate a gene frequency of the colony (G0)

- Genotype a limited number of samples from next generation
  estimate a gene frequency of the colony (G+1)

⋮

- Genotype a limited number of samples from future generation
  estimate a gene frequency of the colony (G+n)

Therefore, it is difficult to determine whether the difference in gene frequency between the samples is due to a sampling error or to an unsuitable mating system.

Genetic monitoring in closed colony animals

New method

- Genotype *all breeders* in nucleus stock (basic generation; G0)

  determine (not estimate) the exact genetic profile of the stock

  ⇓

  definitive method values

- Genotype all or a limited number of samples from future generation (G+n)

  evaluate a differentiation between observed genic frequency and the definitive method values

How should we genotype all breeding pairs ?

Polymorphic markers

- Biochemical markers
- Immunological markers
  require tissue sample (depend on a marker)
  need to sacrifice for obtaining it

- DNA markers (microsatellite marker)
  use only DNA as a sample (for any marker)
  don’t need to sacrifice
  applicable to large scale assay

Microsatellite DNA marker

Short segments of DNA
that have a repeated sequence such as CACACACA

D17Mgh5

AGCATCCATCCACTCCTATACTGCTGCCCTTTATGGAGATGTGCAATGTATAGGAACAAATTGCAGGGCTA

TGGCCATGAAGCAGCAATAATTTGCCTTGCACCATGGTACAACCCCAGGGCACATGTGCGCACACACACA

Forward primer

CACACACACACACACACACACACACACACACACACACACAATTTTCAGTCCACACTCCATTCCTCAAACTATAG

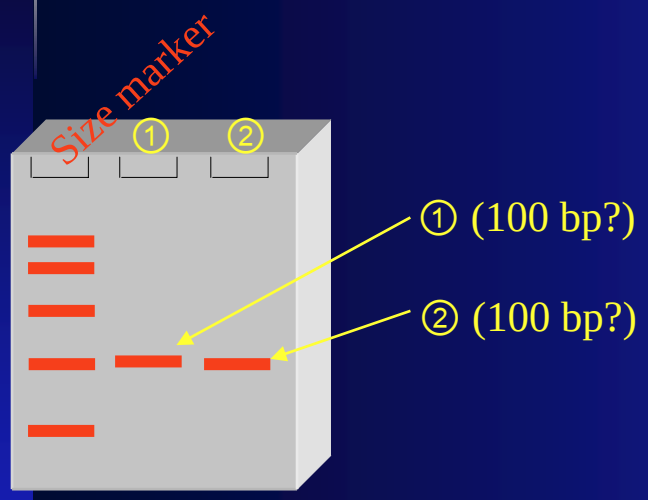
Reverse primer

CACTTTCTTTTAATTAATTGCCTTTGGTCTCTAAATTCAACTCAAGTCTTCACTTTGGAAGTGTCACAGACT

ACCTGGATCATTAT

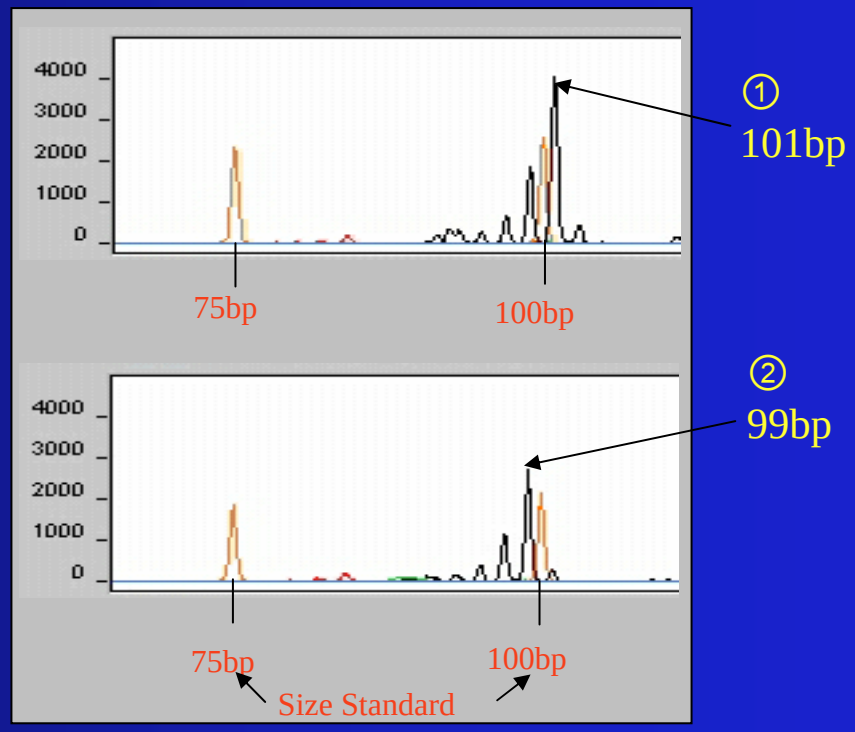
Microsatellite DNA analysis

Agarose gel electrophoresis



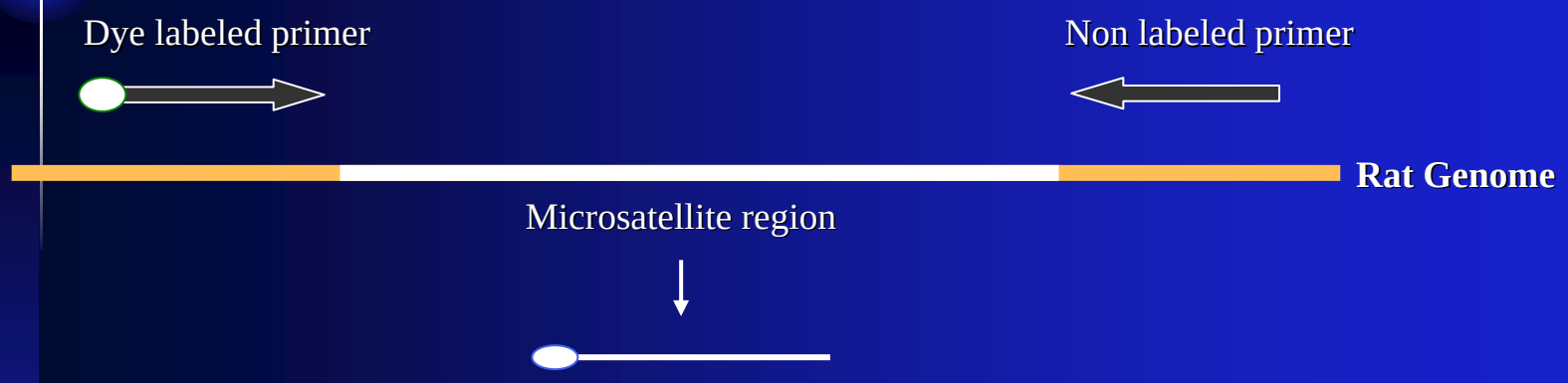
- Calculate with size standard
- Reproducible but low resolution

Capillary electrophoresis



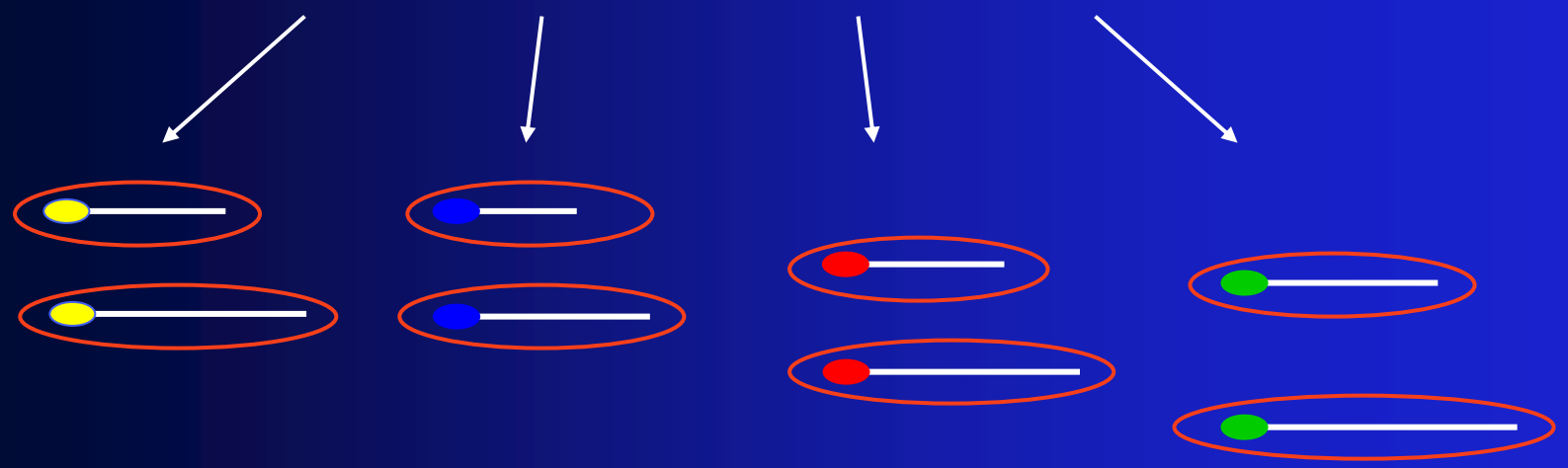
- Automated size calling
- High resolution and reproducibility
- Four fluorescent dye

Multiplexed PCR method

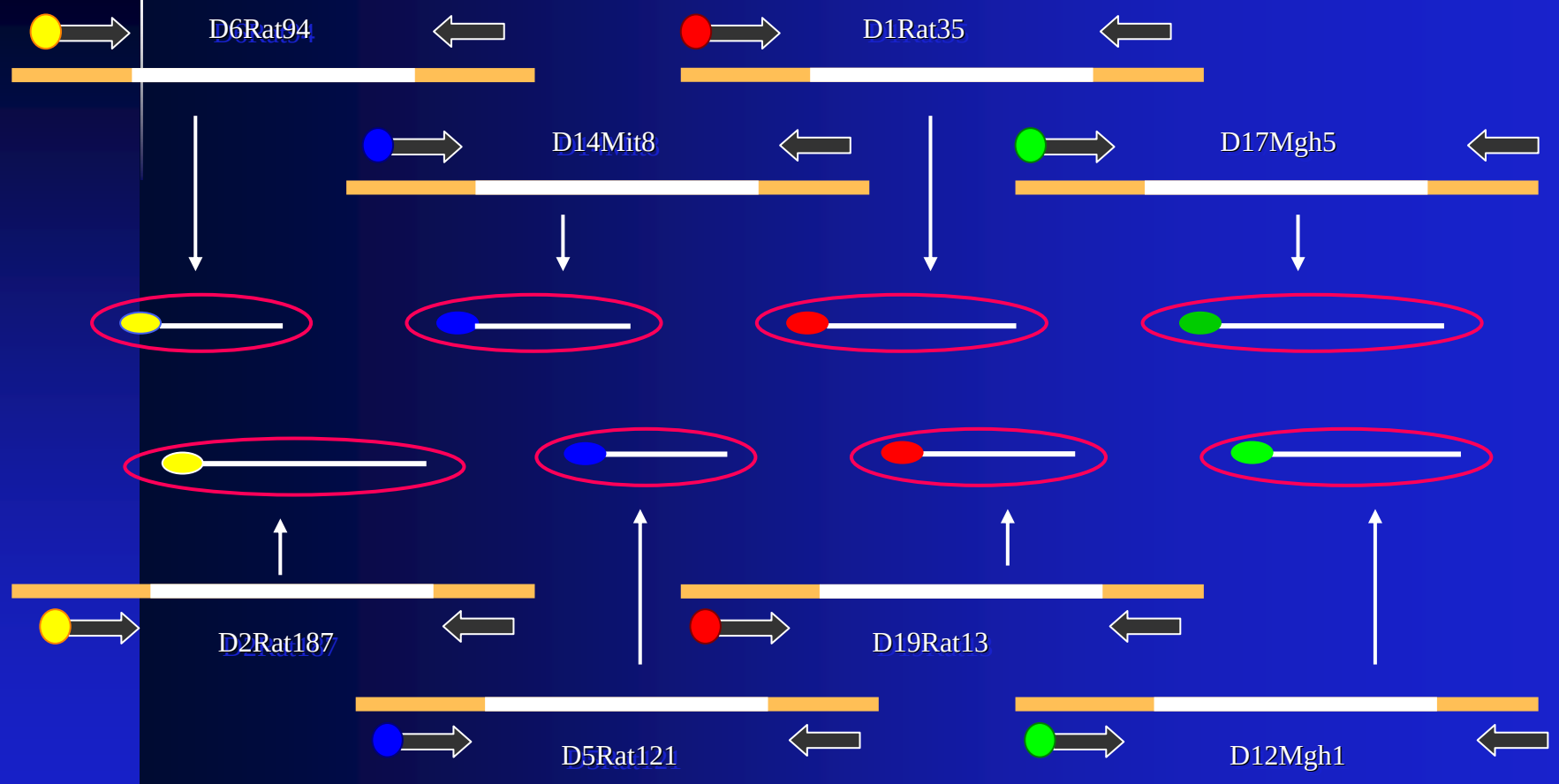


Different color (dye) and size (PCR product)

PCR product

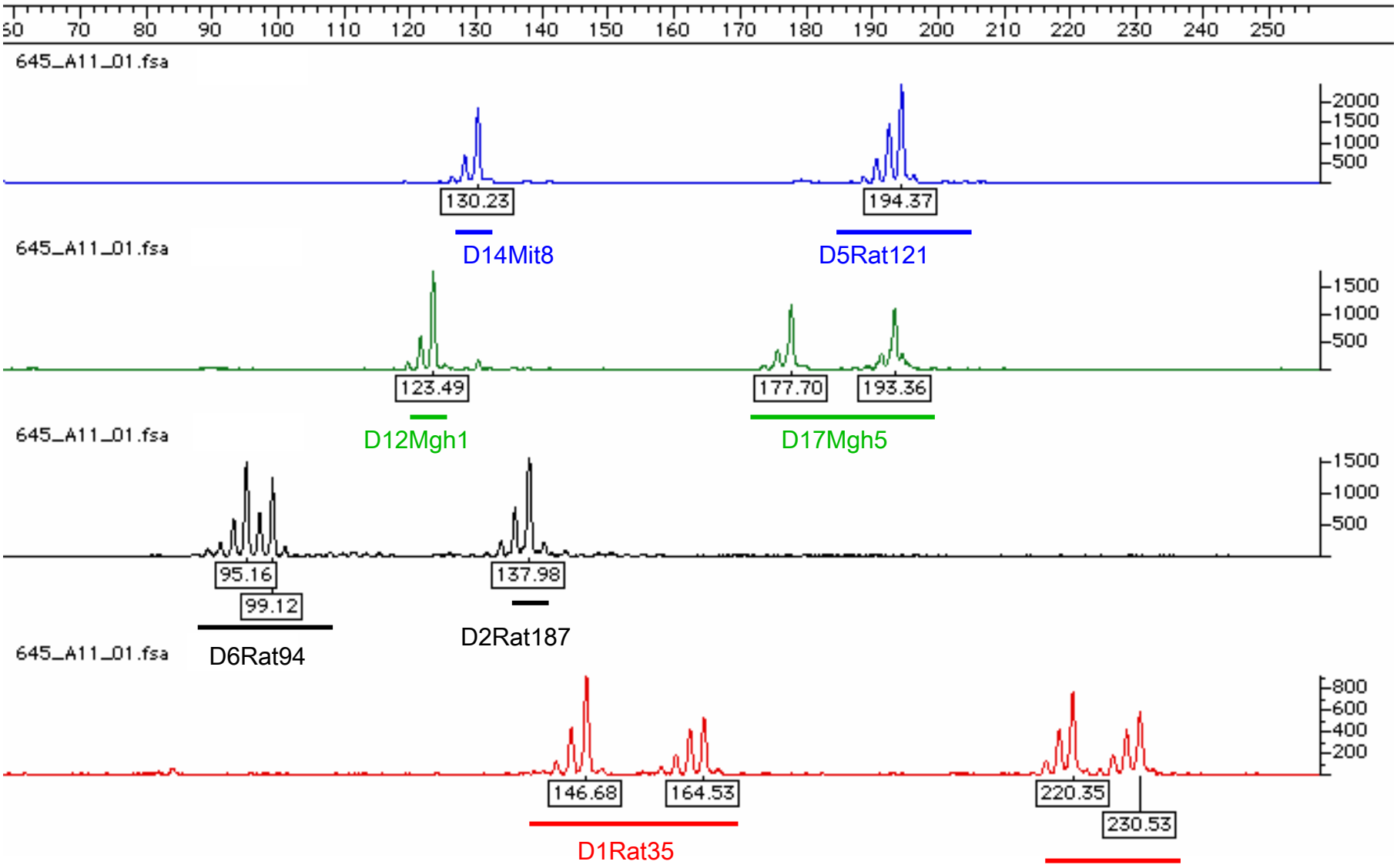


Multiplexed PCR method



Primer mixture: Rat A (for GALAS rat)

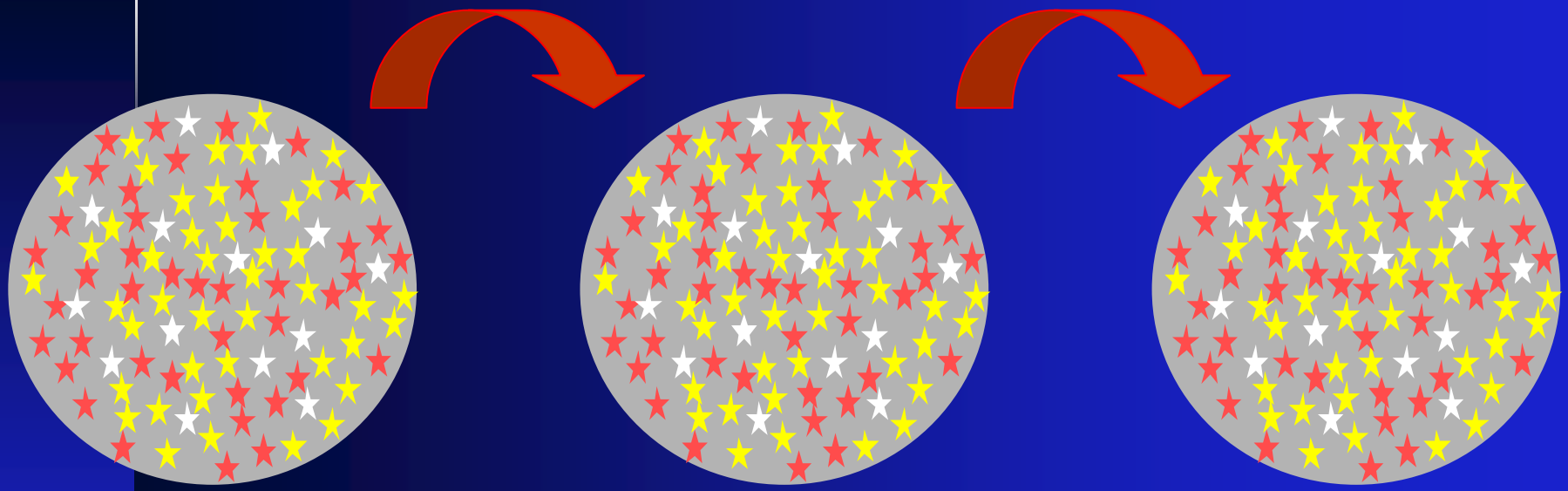
Multiplexed PCR (capillary electrophoresis)



Primer mixture: Rat A (for GALAS rat)

Generational stability of genetic profiles

repopulation *repopulation*



G0 (1,074 rats; 537 pairs) G+1 (1,154 rats; 577 pairs) G+2 (1,170 rats; 585 pairs)

Verification of generational stability (genetic differences)

Evaluation of the effectiveness of new monitoring method

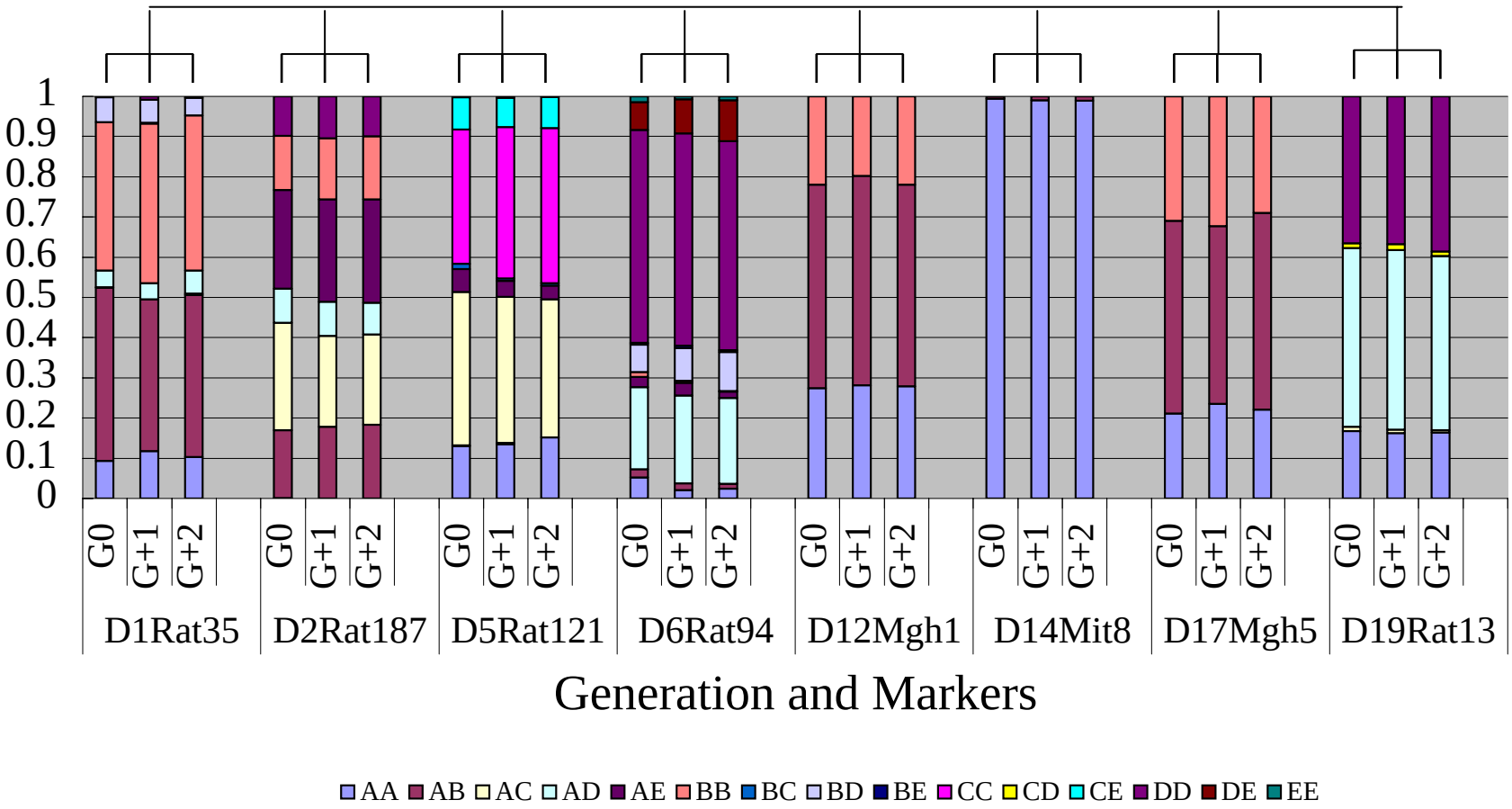
Microsatellite allelic data in GALAS rat breeders

Locus	Population	Genotype															Sample size
		AA	AB	AC	AD	AE	BB	BC	BD	BE	CC	CD	CE	DD	DE	EE	(pairs)
D1Rat35	G0	101	462	2	44	0	396	0	66	0	0	0	0	3	0	0	1074 (537)
	G+1	136	435	1	45	0	459	2	66	0	0	0	0	10	0	0	1154 (577)
	G+2	120	472	4	67	0	451	1	51	0	0	0	0	4	0	0	1170 (585)
D2Rat187	G0	0	1	0	0	0	182	286	263	0	92	144	0	106	0	0	1074 (537)
	G+1	0	0	0	0	0	206	260	293	0	99	176	0	120	0	0	1154 (577)
	G+2	0	0	0	0	0	214	263	301	0	92	184	0	116	0	0	1170 (585)
D5Rat121	G0	140	2	409	0	62	0	14	0	0	358	0	87	0	0	2	1074 (537)
	G+1	155	4	420	0	45	0	8	0	0	434	0	84	0	0	4	1154 (577)
	G+2	178	0	401	0	40	0	7	0	0	451	1	90	0	0	2	1170 (585)
D6Rat94	G0	56	22	0	219	27	14	0	73	4	0	1	0	568	74	16	1074 (537)
	G+1	24	20	0	251	37	5	0	95	7	0	0	0	609	98	8	1154 (577)
	G+2	29	13	0	251	18	1	0	114	5	0	1	0	607	119	12	1170 (585)
D12Mgh1	G0	294	544	0	0	0	236	0	0	0	0	0	0	0	0	0	1074 (537)
	G+1	325	601	0	0	0	228	0	0	0	0	0	0	0	0	0	1154 (577)
	G+2	326	587	0	0	0	257	0	0	0	0	0	0	0	0	0	1170 (585)
D14Mit8	G0	1067	7	0	0	0	0	0	0	0	0	0	0	0	0	0	1074 (537)
	G+1	1143	11	0	0	0	0	0	0	0	0	0	0	0	0	0	1154 (577)
	G+2	1157	13	0	0	0	0	0	0	0	0	0	0	0	0	0	1170 (585)
D17Mgh5	G0	227	514	0	0	0	333	0	0	0	0	0	0	0	0	0	1074 (537)
	G+1	271	510	0	0	0	373	0	0	0	0	0	0	0	0	0	1154 (577)
	G+2	259	572	0	0	0	339	0	0	0	0	0	0	0	0	0	1170 (585)
D19Rat13	G0	180	0	11	477	0	0	0	0	0	1	13	0	392	0	0	1074 (537)
	G+1	188	0	9	516	0	0	0	0	0	0	17	0	424	0	0	1154 (577)
	G+2	191	0	8	505	0	0	0	1	0	0	14	0	451	0	0	1170 (585)

Conserved genotypic frequencies through a generation

Genotypic Frequencies in GALAS rat breeders

No genotypic differentiation was observed.
(Fisher's exact test, df = 16, Chi^2 = 14.1, P-value = 0.588)



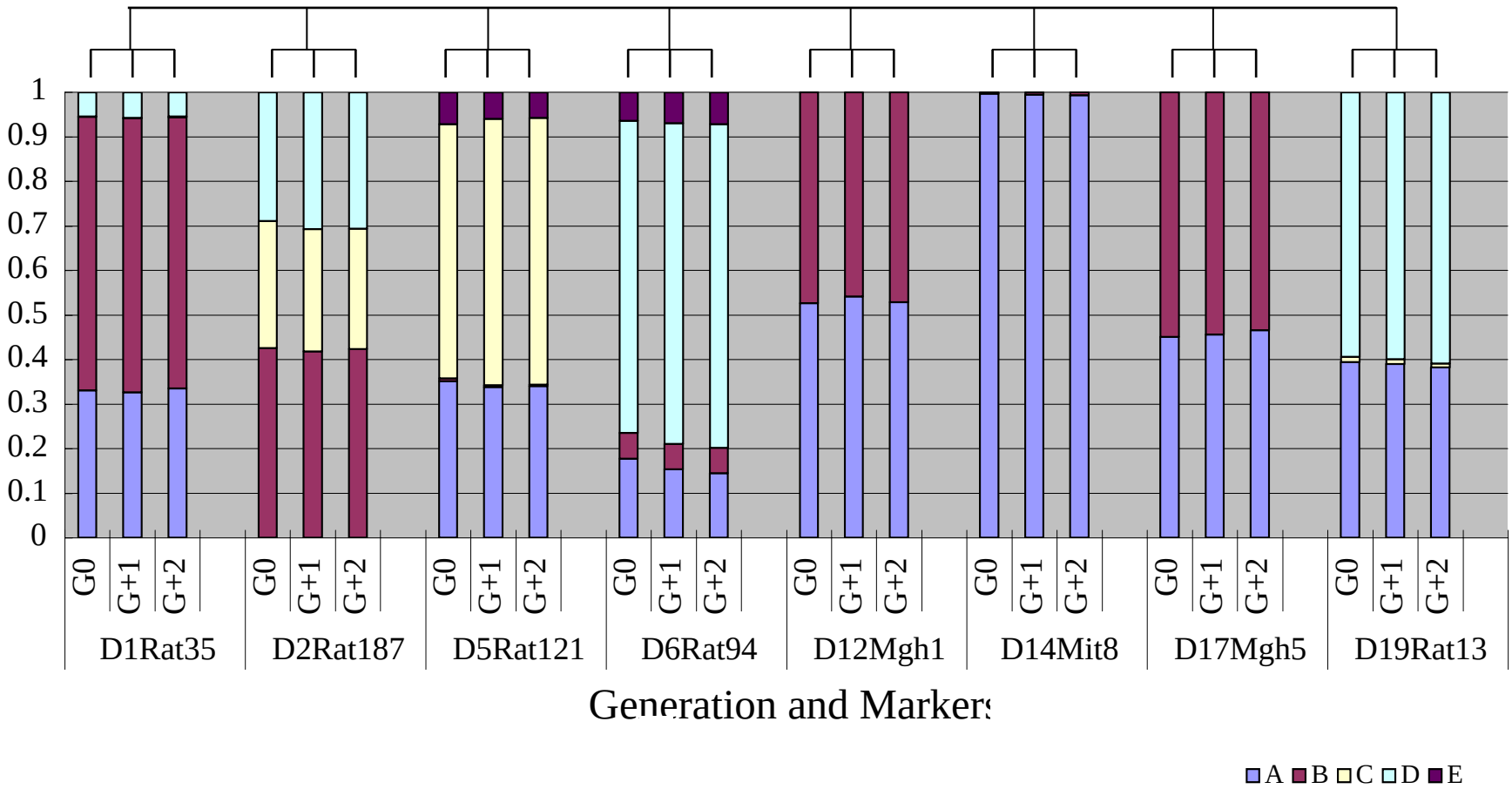
Microsatellite allelic data in GALAS rat breeders

Locus	Population	N	Alleles				
			Allele A	Allele B	Allele C	Allele D	Allele E
D1Rat35			146 bp	164 bp	166 bp	168 bp	
	G0	4	0.331	0.614	0.001	0.054	
	G+1	4	0.326	0.616	0.001	0.057	
	G+2	4	0.335	0.609	0.002	0.054	
D2Rat187			134 bp	136 bp	138 bp	148 bp	
	G0	4	< 0.001	0.425	0.286	0.288	
	G+1	3	N.D.	0.418	0.275	0.307	
	G+2	3	N.D.	0.424	0.27	0.306	
D5Rat121			181 bp	191 bp	195 bp	197 bp	205 bp
	G0	4	0.351	0.007	0.571	N.D.	0.071
	G+1	4	0.338	0.005	0.598	N.D.	0.059
	G+2	5	0.341	0.003	0.599	< 0.001	0.057
D6Rat94			83 bp	95 bp	97 bp	99 bp	103 bp
	G0	5	0.177	0.059	< 0.001	0.7	0.064
	G+1	4	0.154	0.057	N.D.	0.72	0.069
	G+2	5	0.145	0.057	< 0.001	0.726	0.071
D12Mgh1			121 bp	123 bp			
	G0	2	0.527	0.473			
	G+1	2	0.542	0.458			
	G+2	2	0.529	0.471			
D14Mit8			130 bp	132 bp			
	G0	2	0.997	0.003			
	G+1	2	0.995	0.005			
	G+2	2	0.994	0.006			
D17Mgh5			177 bp	193 bp			
	G0	2	0.451	0.549			
	G+1	2	0.456	0.544			
	G+2	2	0.466	0.534			
D19Rat13			220 bp	222 bp	224 bp	230 bp	
	G0	3	0.395	N.D.	0.012	0.593	
	G+1	3	0.39	N.D.	0.011	0.598	
	G+2	4	0.382	< 0.001	0.009	0.608	

Conserved gene frequencies through a generation

Genic Frequencies in GALAS rat breeders

No genic differentiation was observed.
(Fisher's exact test, df = 16, Chi^2 = 14.3, P-value = 0.570



Result

- *We did not find any significant differences among each population in their gene frequency and genotypic frequency.*

Closed colony GALAS rats

- whose gene frequencies will not change from one generation to the next.

Genetic variation

Individual rat has own genetic profile (genotypic variation)

In this study, we are using eight microsatellite markers.

	D1Rat35	D2Rat187	D5Rat121	D6Rat94	D12Mgh1	D14Mit8	D17Mgh5	D19Rat13	PROFILE
G+2-725	BD	BD	AC	AD	BB	AA	AB	AD	BDBDACADBBAAABAD
G0-632	BD	BD	AC	BD	AB	AA	BB	AD	BDBDACBDABAABBAD
G0-250	BD	BD	AC	DD	AB	AA	AA	AD	BDBDACDDABAAAAAD
G+1-705	BD	BD	AC	DD	AB	AA	AB	AD	BDBDACDDABAAABAD
G+1-473	BD	BD	AC	DD	AB	AA	AB	AD	BDBDACDDABAAABAD
G+2-309	BD	BD	AC	DD	AB	AA	AB	AD	BDBDACDDABAAABAD
G+2-723	BD	BD	AC	DD	AB	AA	BB	AA	BDBDACDDABAABBAA

Result 2

Closed colony GALAS rats

- have high levels of genetic variation

- *genotyped all breeding pairs with 8 microsatellite markers*
- *determined exact population genetic structure*

Conclusion

We genotyped all breeders (3,398 rats in consecutive three generations) with 8 microsatellite markers (27,184 data).

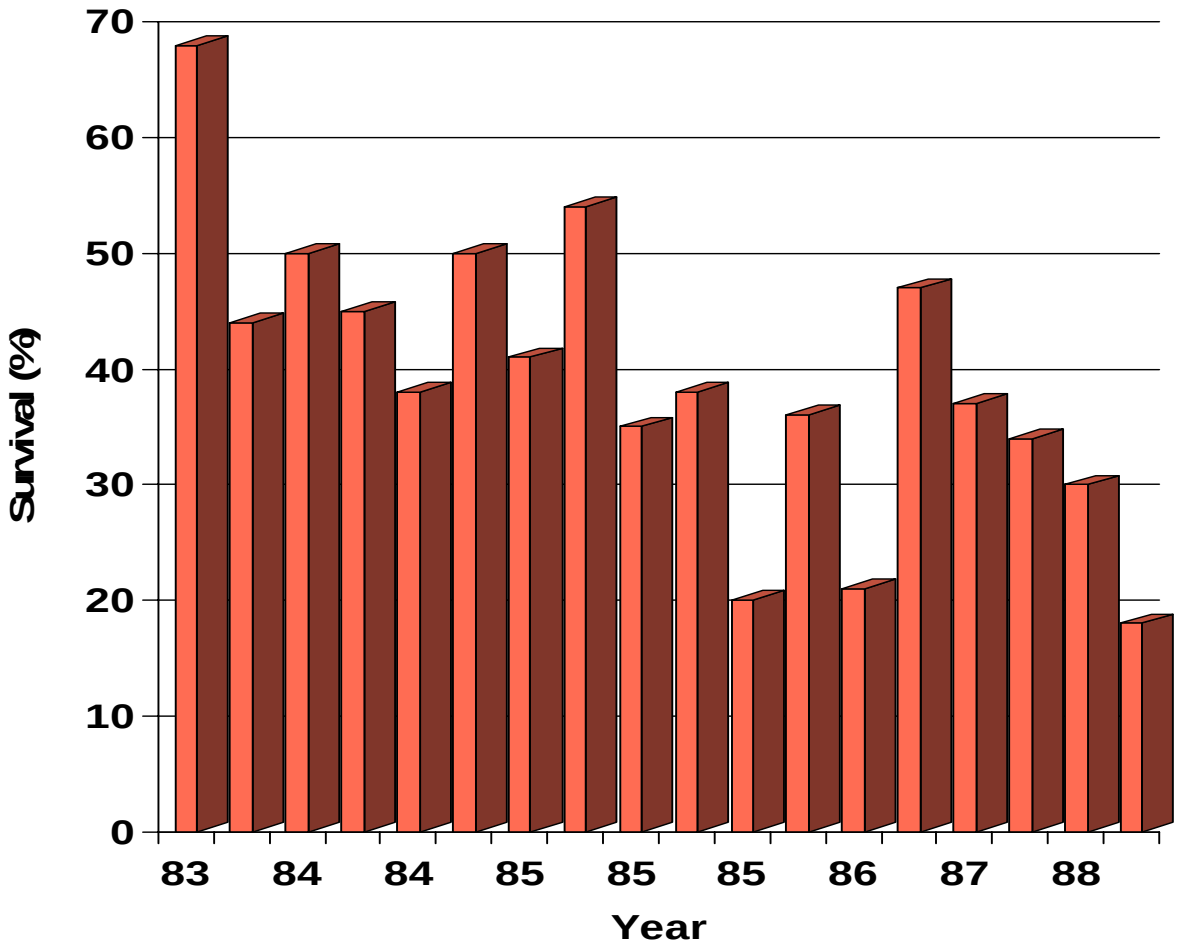
- Closed colony GALAS rat population has high levels of genetic variation

- We did not find any significant differences among each population in their gene frequency and genotypic frequency

- These results suggested that the GALAS rat colony was able to be repopulation conserving their genetic profiles, and *the genetic divergence was able to be verified by deviating from the definitive method values*

Example 1: Trend Towards Reduced Longevity Survival

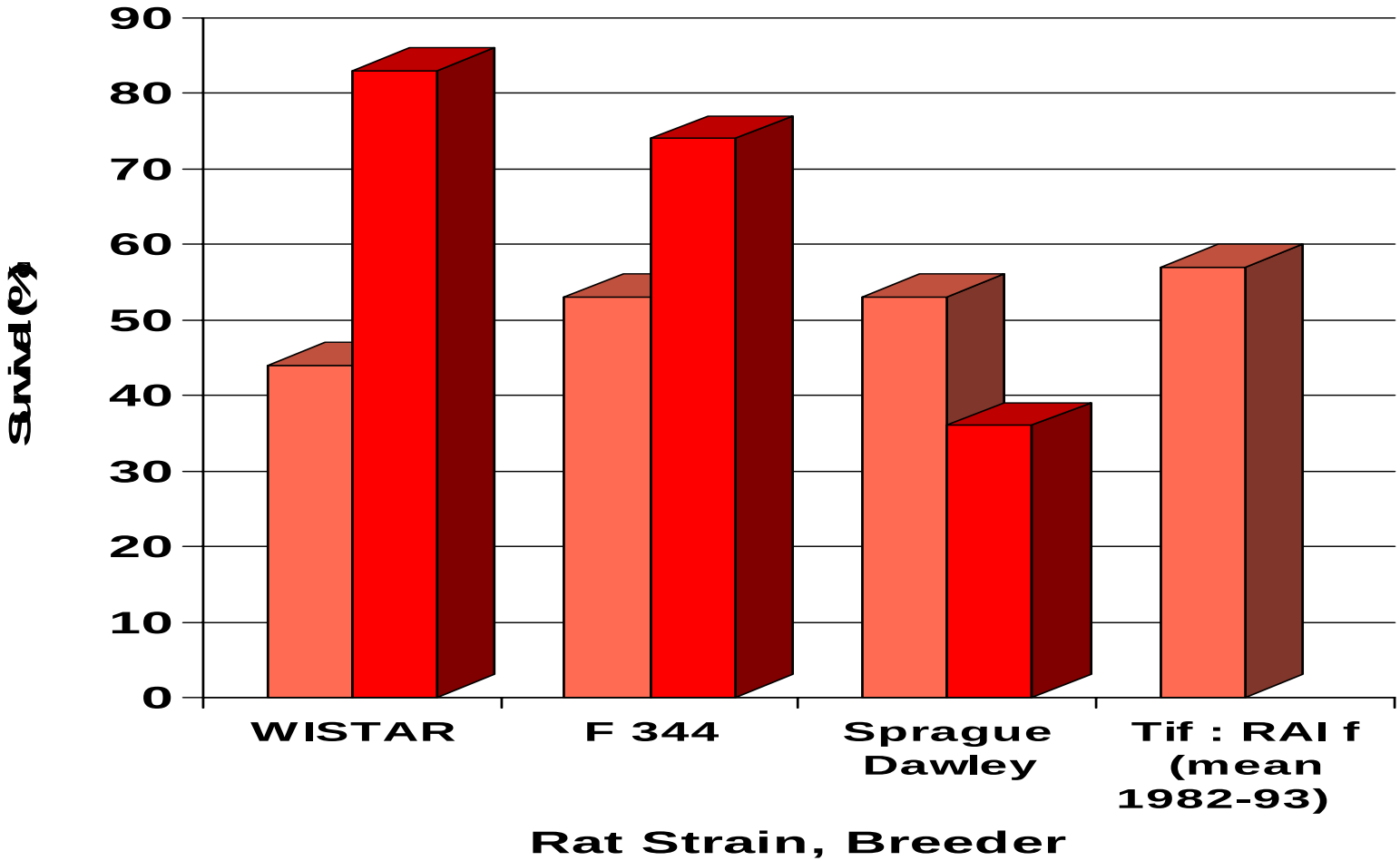
Sprague Dawley Rats (male) at Week 104



NOVARTIS

Example 1: Longevity of Rats by Strain and Breeder

Survival of Male Rats at Week 104



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Example 2a: Drug ABC

Sprague-Dawley Rat 1 month Toxicology Study - *gavage* (15/group/sex)

Study Location - Company X Facility in Japan

Doses (mg/kg)	0	50	150	450
Clinical signs	-	-	unkept, urinary staining	mortality (5m 3f) urinary staining
Clinical Chem	-	Sporadic inc AST/ALT	AST/ALT (2X)	AST/ALT 2-4X AlkP, Bil inc
Pathology	-	-		
liver			focal necrosis (3m)	necrosis (3m)
heart				inc weight (10% /BW)
AUC ngXh/ml		35	80	550

Conclusion: ***target organs*** ***> liver***
> possible heart

linear drug exposure

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Example 2a: Drug ABC

Sprague-Dawley Rat 3 month Toxicology Study - *Dietary Admix* (20/group/sex)

Study Location - Company X Facility in Japan

Doses (mg/kg)	0	15	40	120
Clinical signs mortality	-	-	unkept,	urinary staining (3m, 2f)
Clinical Chem	-	-	ALT (<2X)	AST/ALT 2-4X
Pathology	-	-		
liver			focal necrosis (3m, 1f)	focal necrosis (3m; 4f)
heart	-	-	-	(10-25% /BW M&F)
AUC ngXh/ml		15	45	180

Conclusion ***target organs*** ***> liver***
> heart
linear drug exposure (similar to gavage)
MTD for carc. study 40/mg/kg

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Example 2a: Drug ABC

Sprague-Dawley Rat 6 month Toxicology Study - *Dietary Admix* (20/group/sex)

Study Location - CRO EU

Doses (mg/kg)	0	10	30	60
Clinical signs			urinary staining	urinary staining
mortality	1m	1f	2m, 1f -	4m 6f
Clinical Chem	-	-	BUN inc	BUN inc
Pathology				
liver	min focal necrosis all groups			
renal	-	-	focal tubular nec. m>f	tub. & glomerular nec. (7m; 6f)
heart	-	-	-	(10-15% /BW M&F)
AUC ngXh/ml		7	25	50

Conclusion: ***target organs*** ***> kidneys***
> heart
No significant liver effects!

linear drug exposure
MTD for carc. study 30/mg/kg

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Example 2a: Drug ABC

Proposed Carcinogenicity Study to FDA

Carcinogenicity Study Proposal

- **Sprague-Dawley rat**, 2 years
- US CRO
- Dietary administration: 0, 0, 5 15, 45 mg/kg
- Based on MTD - from 3 month toxicity study - dose limiting hepatic and CV effects (CV known for class to increase in severity)

CAC Response

- **No concurrence**
  - Toxicity profile and sensitivity changed with changing study sites
  - No *hepatic* effects in EU-based study; No *renal* effect in Japan-based study,
  - CV effects not exposure or duration of treatment related across studies
  - Exposure by dietary administration variable by study site
  - No dose ranging at US facility
- Response may be influenced by animal source, husbandry, or diet

- **CONSEQUENCE**
  - **1 year delay in conduct of Carcinogenicity Study to repeat DR in US CRO**

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Example 2b:
Antipsychotic Drugs and Proliferative Lesions and Findings in Carcinogenicity Studies

Antipsychotic Drug	Tested in strain	Rat		Mouse	
		Male	Female	Male	Female
1	Rat: Sprague-Dawley Mouse: CD-1	Mammary gland hyperplasia		Mammary gland hyperplasia	Mammary gland hyperplasia
2	Rat: Wistar Mouse: Swiss Albino	Thyroid gland follicular adenoma	Mammary gland adenocarcinoma	Thyroid gland follicular adenoma	
3	Rat: Wistar Mouse: Swiss Albino	Endocrine pancreas adenoma Mammary gland adenocarcinoma	Mammary gland adenocarcinoma		Pituitary adenoma Mammary gland adenocarcinoma
4	Rat: Long-Evans Mouse: CD-1				Pituitary gland adenoma Pituitary gland carcinoma Mammary gland adenocarcinoma
5	Rat: Fischer 344 Mouse: not specified		Mammary gland adenoma Mammary gland adenocarcinoma		Liver hemangioma Liver hemangiosarcoma (not repeated in a second study) Mammary gland adenoma Mammary gland adenocarcinoma

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Example 3:
Phenolphthalein Induces Thymic Lymphomas Accompanied by Loss of the p53 Wild Type Allele in Heterozygous p53-Deficient (±) Mice

- Heterozygous p53-deficient (±) female mice on a **C57BL/6 background**
- Phenolphthalein treatment for 6 months – feed consumption
- Carcinogenic response in thymus of heterozygous p53-deficient (±) mice after 4 months
- Phenolphthalein induced complete loss of the wild type p53 allele sequence in thymic tumor tissue, but not in normal tissue specimens (ear or kidney)

Dunnick JK et al, *Toxicologic Pathology* **25** (1997) 533-540

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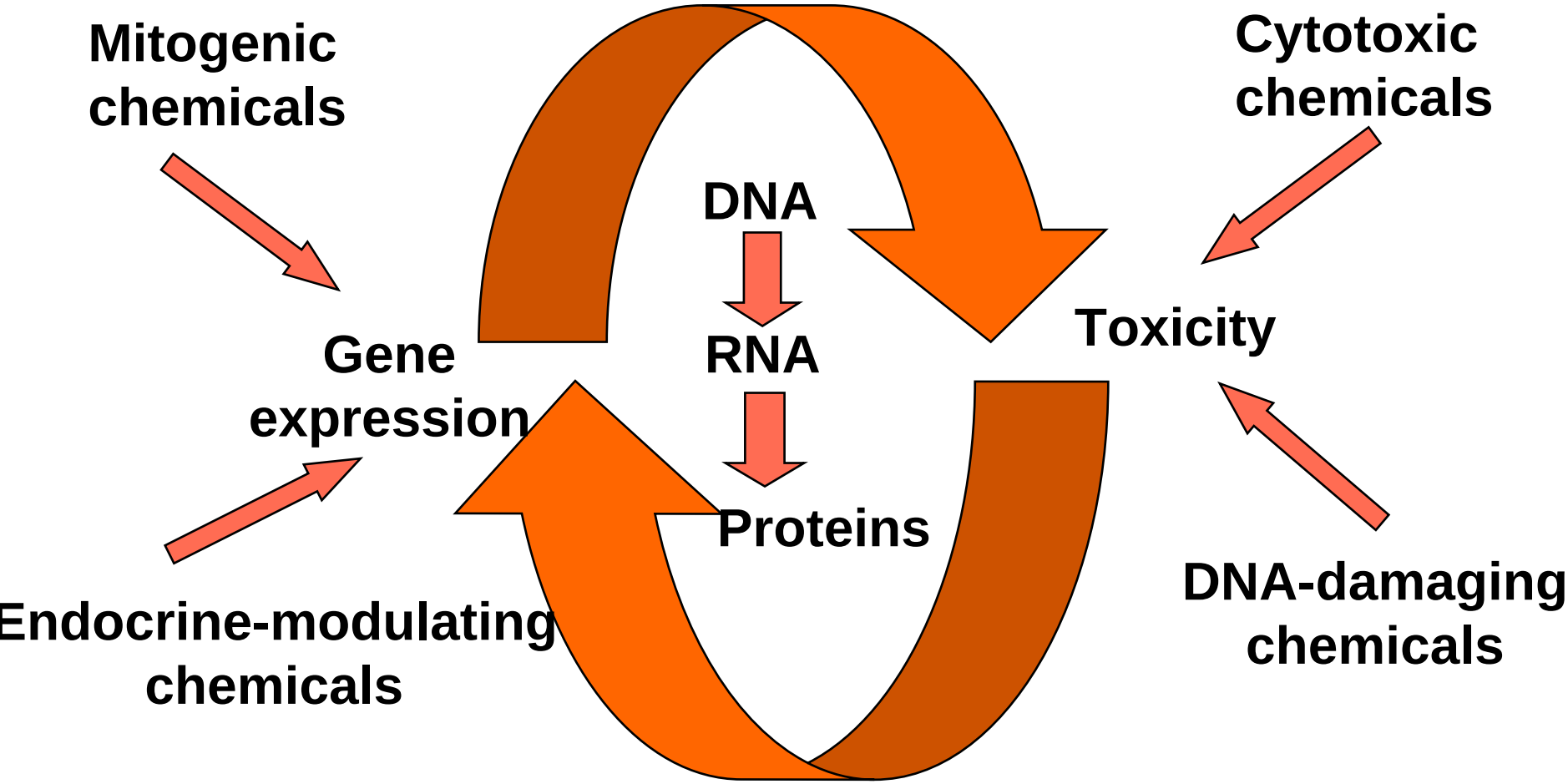
Relationship between gene expression and toxicity



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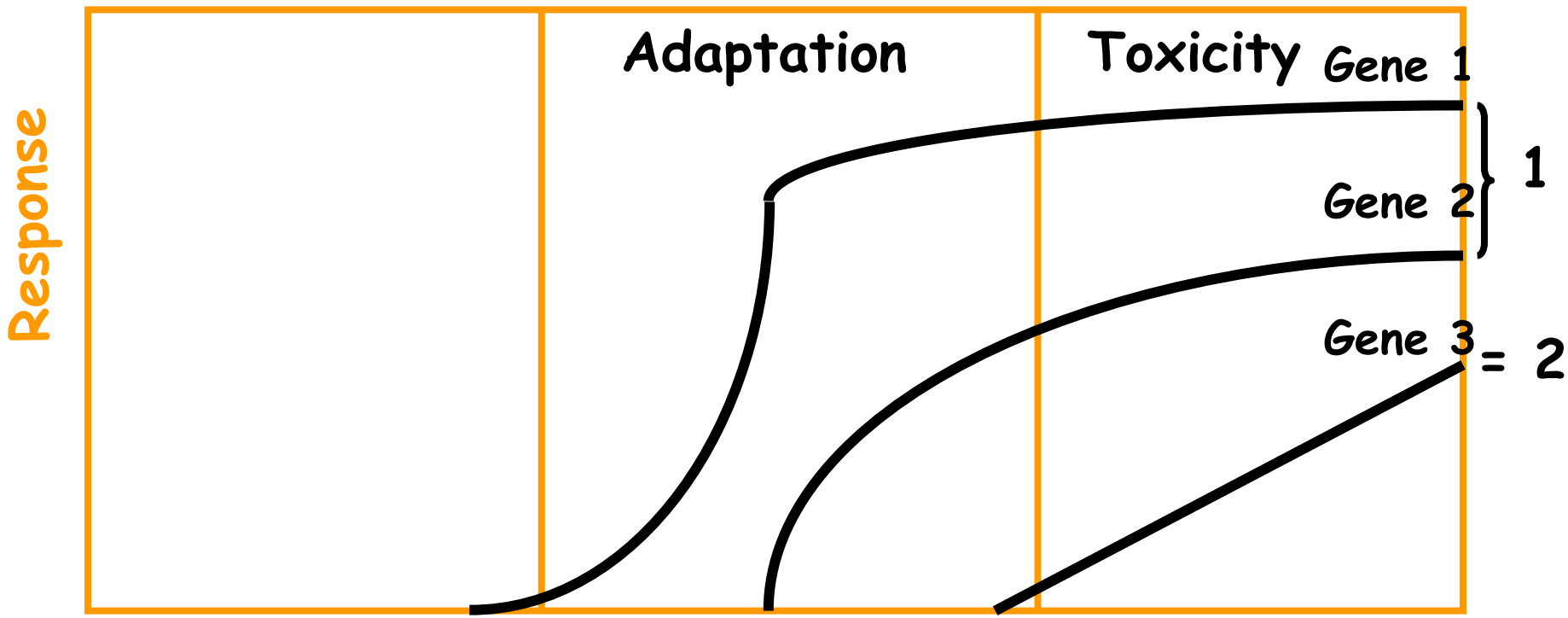
Relationship between gene expression and toxicity



1 = linked to adaptive response
2 = linked to toxicity

Refs.: Chemical Industrial Toxicology.website (CIT) & TRENDS in Pharmacol. Sci. 22, 6, 285-291, 2001

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An Emerging Issue: Toxicogenomics

Strengths

identify genetic strain differences in response

explain differences in pathophysiology based on differential genetic expression profile

Weaknesses

incomplete validation

current non-GLP compliance

Opportunities

Sensitivity of toxicogenomics will widen the observed differences between outbred strains used for regulatory pharmaceutical studies, thereby driving a global standardization and control of outbred rodent strains in order to counteract the above tendency

Threats

quality standards of sampling methodology influences quality and interpretability of results heavily (depending on complexity of organ / tissue)

(patho)physiological ‘noise level’ changes need to be differentiated from (dose-dependent) drug-related effects

(dose-dependent) drug-related response profiles may differ across strains and therefore require careful analysis interpretation to arrive at a biologically meaningful and integrated understanding of the drug responses (adaptive vs toxic)

Current Situation in Toxicogenomics

- In the regulatory setting there is minimal information on genetics of rodents used in assays
- genetics is considered as important regulatory information for genetically engineered test species, primarily in relation to quality control of response element (e.g. Loss of critical palindromic transgene promoter sequence in chemically induced Tg.AC mouse skin papillomas expressing transgene-derived mRNA; Thompson et al, *Mol Carcinog* **32** (2001) 176)
- There are emerging areas that call for evaluation of the effects of rodent genetic divergence on study interpretation
- Future approach is to utilize toxicogenomics for a new mechanistic understanding of target organ toxicity

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