



Genetic evaluation of F₂ inbred strain of Swiss albino mice by microsatellite markers

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ABSTRACT

Genetic characterization of F₂ inbred Swiss albino mice was done using 11 microsatellites markers (D1Mit15, D2Mit51, D2Mit61, D3Mit15, D3Mit55, D5Mit18, D7Mit323, D8Mit14, D9Mit27, D10Mit180, and D11Mit167). The results indicated that genotypic frequencies at all the loci were in Hardy-Weinberg disequilibrium ($P < 0.001$). Total number of alleles per locus ranged from 3 (D2Mit61, D3Mit15, D8Mit14, D9Mit27, D10Mit180, D11Mit167) to 4 (D1Mit15, D2Mit51, D3Mit55, D5Mit18, D7Mit323), with a mean of 3.45. The numbers of effective alleles ranged between 1.847 and 3.814. The observed heterozygosity (H_o) was maximum for D1Mit15 (0.660) and minimum for D5Mit18 (0.080), with mean of 0.269. The F_{IS} estimates was ranged from 0.0817 (D1Mit15) to 0.8799 (D5Mit18). The average inbreeding coefficient was 0.592, which indicates that parents were more related than expected under random mating. The range of PIC value (from 0.414 to 0.689) for various microsatellite loci was revealing that population under investigation was of high diversity maintaining a multiple allele.

Keywords: Genetic characterization, Inbred strain, Inbreeding coefficient, Microsatellite marker, Swiss albino mice

The laboratory mice are widely used as a pre eminent research model in the various field of biological research. Mice are prized for many qualities, including their small size, short generation time, and ease of breeding within the laboratory and low maintenance cost (Baumans 2004, Danneman *et al.* 2012). The importances of inbred mice are well recognized in various biological researches, throughout the world because of their attributes of unique and identical genotypes and phenotypes. An inbred strain provides more power and requires fewer animals per experiment by limiting the noise caused by segregating genetic variation (Casellas 2011, Danneman *et al.* 2012).

The genetic monitoring of inbred strain of mice has become an essential component of mouse colony management for detecting any kind of genetic contamination (Nomura *et al.* 1984). Microsatellites are highly polymorphic tract of repetitive DNA in which certain DNA motifs of 1–6 base pairs (bp) in length are repeated, typically 5–50 times (Gulcher *et al.* 2012), considered as ideal tools for deciphering genetic variability and contamination due to their abundance, random distribution throughout genomes, and high degree of polymorphisms (Gulcher *et al.* 2012).

Inbreeding depression exists essentially in all populations and for almost all characters (Falconer and Mackay 1996).

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The estimation of inbreeding coefficient by using a panel of microsatellite markers approach is more convenient and more reliable than conventional pedigree based approach (Selvaggi *et al.* 2010). Microsatellite approach exploits the fact that inbreeding decreases heterozygosity, therefore, it can be used to estimate inbreeding coefficient of an individual and furthermore, to estimate inbreeding level of a population (Naghavian *et al.* 2016).

Therefore, considering the importance of genetic monitoring of inbred strain of mice, the present study was undertaken with the objective to genetic evaluation of F₂ inbred strain of Swiss albino mice for various population genetic parameters, using a panel of microsatellite markers.

MATERIALS AND METHODS

Experiment population and sample collection: A population of F₂ inbred Swiss albino mice was produced through mating of 64 pairs of F₁ inbred Swiss albino mice at Laboratory Animal Research (LAR) Section, Animal Genetics Division, ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly. A total of 100 F₂ inbred Swiss albino mice were randomly selected for the present investigation. These mice were reared under similar feeding and managerial conditions throughout the experimental period. About 1–1.5 cm tail tissues were collected from 100 adult F₂ inbred Swiss albino mice in a sterile 15 ml polypropylene centrifuge tube and kept on ice until transferred to the laboratory.

DNA extraction: Genomic DNA isolated from the tail tissue of mice using DNA Isolation kit as per the protocol (Qiagen DNeasy Blood & Tissue Kit). DNA concentration and purity (A260/A280 ratio) for each sample was assessed using a spectrophotometer and by 0.8% agarose gel electrophoresis. The measured DNA samples were stored at -80°C until further analysis.

PCR-microsatellite analysis: Eleven microsatellite loci [D7Mit323 (Basta *et al.* 2004); D1Mit15, D2Mit51, D3Mit55, D11Mit167 (Zhang *et al.* 2007); D3Mit15, D10Mit180 (Shang *et al.* 2009); D2Mit61, D5Mit18, D9Mit27 (CCMB website); D8Mit14 (Zuo *et al.* 2012)] were taken in the present study. Eleven sets of forward and reverse loci-specific oligonucleotide primers were taken from literature. Detailed information about microsatellite loci along with primer sequence, annealing temperature, and amplicon size of each locus is given in Table 1. The working solutions of both forward and reverse primers were prepared to obtain a final concentration of 10 pmol of each primer. Final reaction mix (25 μl) comprised of Forward primer (1.0 μl), Reverse primer (1.0 μl), Dream Taq Green buffer (2.5 μl), dNTPs mix (0.5 μl), Taq polymerase (0.2 μl), DNA template (2 μl) and Nuclease Free Water (17.8 μl). PCR was carried out in a 25 μl reaction volume, which was kept constant for all reactions using thermo cycler (Bio-Rad, USA).

The optimization of appropriate annealing temperature with respect to each primer was determined by gradient PCR. The PCR conditions involved initial denaturation at 95°C for 5 min, followed by 40 cycles with denaturation at 94°C for 1 min, annealing temperature ranges from 52.5°C and 62.0°C for 45 sec to specifically amplify a target region 1 and 2 respectively, extension at 72°C for 1 min followed by a final extension at 72°C for 5 min. For microsatellite genotyping, the amplified products were first run on 2.5% (w/v) agarose gel electrophoresis to check for their amplification. The products were then resolved on ultra-high-resolution agarose (4%; at 50–75 V; for 3–6 h) to differentiate alleles as per their length (in base pairs). Visualizing of each gel was done under Gel Doc (Genesnap, Syngene) system and allele size was determined by using Gel Analyzer (2010) software.

Statistical analysis: Observed and expected heterozygosity, allele frequencies, observed number of alleles per locus, effective number of alleles, polymorphic information content (PIC), allelic diversity, F-statistics and Hardy Weinberg equilibrium were estimated using POPGENE 32 (Yeh *et al.* 1999) software. The inbreeding coefficient (F) was calculated as the deviation of the observed heterozygosity of an individual relative to the heterozygosity expected under random mating (Lukas and Donald, 2002) which was derived as:

$$F_{IS} = 1 - (H_o/H_e)$$

where F, coefficient of inbreeding; H_o , observed frequency of heterozygous individuals; H_e , expected frequency of heterozygous individuals in the population.

Table 1. Details of microsatellite loci along with primer sequence, annealing temperature (AT), and amplicon size (AS) of each locus

Loci	Primer sequence (5' → 3')	AT ($^{\circ}\text{C}$)	AS (bp)	References
D1Mit15-FD1Mit15-R	TCCACAGAACTGTCCCTCAAATACACTCACACCACCCCGT	55.0	154–188	Zhang <i>et al.</i> 2007
D2Mit51-FD2Mit51-R	GTGAGGGGTCAATGCCACGGCTCAGTTGTAAAGCACAAAGG	57.5	170–190	Zhang <i>et al.</i> 2007
D2Mit61-FD2Mit61-R	AAAGTCAACTGCTTTCAGTTACCCACAGAAAGTGCCCTTGCATA	55.5	122–158	CCMB website
D3Mit15-FD3Mit15-R	AATTGCATTCCAGGACACAGGAAGTGACGTTGGGTTTG	59.0	143–210	Shang <i>et al.</i> 2009
D3Mit55-FD3Mit55-R	CTGGGACCACCAAGTAGTACCATCAGGACTGCAACTGAGGC	57.5	116–144	Zhang <i>et al.</i> 2007
D5Mit18-FD5Mit18-R	CTGTAGTGGGTGTTTAAATGATGATGCCACTGGTGCTCTCTG	58.0	220–238	CCMB website
D7Mit323-FD7Mit323-R	TTTCACCTTCTAATCCTACTTCTGTGTCCAGAACAGGAATAGATACC	60.0	125–178	Basta <i>et al.</i> 2004
D8Mit14-FD8Mit14-R	TTTTCACACTCACGTGTGCGGTCTCTCTCTCTGCGCGCTG	59.5	130–167	Zuo <i>et al.</i> 2012
D9Mit27-FD9Mit27-R	TAGATTTCTCAGGCAAGGAAGCTCCACTCTGAGCTTGGTGG	58.0	158–194	CCMB website
D10Mit180-FD10Mit180-R	GACCTTCCTTTATACAAAGTCATAGCGTGGTACAGAACTTAGGTGTTTAATTG	58.5	108–206	Shang <i>et al.</i> 2009
D11Mit167-FD11Mit167-R	TCGGATGCTAAGGAAATTCGACACACTCAGTGTGACCTCTGG	57.5	134–150	Zhang <i>et al.</i> 2007

RESULTS AND DISCUSSION

In the present study, 100 F_2 inbred Swiss albino mice were genotyped by PCR-Microsatellite analysis technique for 11 loci. All genotyped loci were polymorphic. The representative image of gel electrophoresis of a microsatellite (D1Mit15) locus in 4% agarose gel is shown in Fig 1. Allelic frequency distribution at 11 microsatellite loci of F_2 inbred Swiss albino mice population is shown in Table 2. A total of 38 alleles were detected for the 11 microsatellite markers. The total number of alleles per locus ranged from 3 (D2Mit61, D3Mit15, D8Mit14, D9Mit27, D10Mit180, D11Mit167) to 4 (D1Mit15, D2Mit51, D3Mit55, D5Mit18, D7Mit323) in F_2 population with a mean of 3.45 which showed all 11 loci were polymorphic and the number of effective alleles ranged between 1.847

and 3.814. These findings were in agreement with Shang *et al.* (2009), however, they observed that BJ and SH population of Kunming mice had 13 and 14 polymorphic loci out of 15, respectively. Zuo *et al.* (2012) observed that 10 out of 42 (23.8%) microsatellite loci showed changed from monomorphism to polymorphism in 29 Knock out (KO) inbred mouse strain. Tarang (2018) observed that number of alleles at 10 microsatellite loci varies from 3 (D2Mit61, D3Mit55, D8Mit14, D9Mit27, D10Mit180, D11Mit167) to 4 (D1Mit15, D2Mit51, D5Mit18, D7Mit323) in both the population (foundation and F_1 inbred) with a mean of 3.40.

The genotypic frequencies of all the microsatellite loci in F_2 inbred mice population were in Hardy-Weinberg disequilibrium ($P < 0.001$). Shang *et al.* (2009) and Tarang (2018) observed similar results in 2 Kunming mice



Fig. 1. PCR-Microsatellite profile of D1Mit15 locus in Swiss albino mice of F_2 inbred generation (4% Metaphor gel electrophoresis). Lane M¹: 100 bp marker, Lane M²: 50 bp marker, Lanes 1–20; Resolved PCR products (Animal no. 1 to 20).

Table 2. Allelic frequency distribution at 11 microsatellite loci in F_2 inbred population of Swiss albino mice

Locus	Allele	Population size (N)	Frequency	SE	Locus	Allele	Population size (N)	Frequency	SE
D1Mit15	205	68	0.340	0.037	D7Mit323	128	41	0.205	0.036
	190	59	0.295	0.030		112	64	0.320	0.043
	175	52	0.260	0.031		106	69	0.345	0.042
	160	21	0.105	0.024		100	26	0.130	0.031
D2Mit51	156	35	0.175	0.029	D8Mit14	160	62	0.310	0.041
	144	48	0.240	0.034		150	103	0.515	0.045
	136	66	0.330	0.041		144	35	0.175	0.036
	128	51	0.255	0.035		190	61	0.305	0.043
D2Mit61	168	46	0.230	0.032	D9Mit27	178	120	0.600	0.045
	156	64	0.320	0.040		158	19	0.095	0.028
	146	90	0.450	0.046	D11Mit167	144	34	0.170	0.031
D3Mit15	160	33	0.165	0.036		128	102	0.510	0.045
	150	141	0.705	0.042		122	64	0.320	0.042
	126	26	0.130	0.030	D8Mit14	160	62	0.310	0.041
D3Mit55	162	29	0.145	0.033		150	103	0.515	0.045
	148	84	0.420	0.046		144	35	0.175	0.036
	142	57	0.285	0.042	D10Mit180	224	64	0.320	0.039
D5Mit18	136	30	0.150	0.034		158	80	0.400	0.041
	246	78	0.390	0.047		138	56	0.280	0.037
	238	74	0.370	0.047					
	232	42	0.210	0.038					
	220	6	0.030	0.017					

population and in the foundation stock of F_1 inbred Swiss albino mice population, respectively. In the global test of deviation from Hardy-Weinberg equilibrium (HWE), the deviations from the expected value may be due to a variety of causes: population subdivision owing to genetic drift, artificial selection, inbreeding, the excess of heterozygote individuals than homozygote individuals, migration, high mutation rate in microsatellite and artificial selection in a breed (Lawson *et al.* 1989; Naghavian *et al.* 2016).

The locus D1Mit15 had 9 genotypes (205/205, 205/190, 205/175, 190/190, 190/175, 190/160, 175/175, 175/160 and 160/160) with genotypes 190/160 and 160/160 having minimum genotype frequency 0.040 whereas genotype 205/190 having maximum genotypic frequency of 0.240. However, Tarang (2018) reported that genotype 190/190 had the highest genotypic frequency in both F_0 (0.350) and F_1 (0.333) mice population, respectively. The loci D2Mit51 had 10 genotypes (156/156, 156/144, 156/136, 156/128, 144/144, 144/136, 144/128, 136/136, 136/128 and 128/128) with genotype 136/128 having minimum genotype frequency 0.030 whereas genotype 136/136 having maximum genotypic frequency of 0.230. However, genotypes (156/136, 144/128 and 136/136) were observed equally with highest genotypic frequency (0.190) in F_0 and genotype 128/128 had highest frequency (0.333) F_1 inbred mice population (Tarang 2018).

The locus D2Mit61 had 6 genotypes (168/168, 168/156, 168/146, 156/156, 156/146 and 146/146) with genotype 156/146 having minimum genotype frequency 0.030 whereas genotype 146/146 having maximum genotypic frequency of 0.390. However, genotype 146/146 was reported with highest frequency in F_0 (0.390) and F_1 (0.550) population of mice (Tarang 2018). The locus D3Mit15 had 5 genotypes (160/160, 160/150, 150/150, 150/126 and 126/126) with genotype 160/150 having minimum genotypic frequency 0.030 whereas genotype 150/150 having maximum genotypic frequency of 0.650. The locus D3Mit55 had 10 genotypes (162/162, 162/148, 162/142, 162/136, 148/148, 148/142, 148/136, 142/142, 142/136 and 136/136) with 3 genotypic 162/142, 162/136 and 148/136 having minimum genotypic frequency 0.010 whereas genotype 148/148 having maximum genotypic frequency of 0.370. While contrary to present studied population, genotype 142/142 was observed with highest genotypic frequency in foundation population (0.470) and in F_1 (0.517) inbred mice population (Tarang 2018).

The locus D5Mit18 had 6 genotypes (246/246, 246/232, 238/238, 238/232, 232/232 and 220/220) with genotype 220/220 having minimum genotypic frequency 0.030 whereas genotype 246/246 having maximum genotypic frequency of 0.370. However, genotypes 220/220 and 238/220 were observed with highest frequency in F_0 (0.310) and in F_1 (0.317) inbred mice population, respectively (Tarang 2018). The locus D7Mit323 had 9 genotype (128/128, 128/112, 128/106, 112/112, 112/106, 112/100, 106/106, 106/100 and 100/100) with genotype 112/100 having minimum genotypic frequency 0.010 whereas genotype

112/112 and 106/106 having maximum genotypic frequency of 0.260. However, genotype 100/100 had the highest frequency in both populations studied with the value of 0.270 in F_0 and 0.283 in the F_1 inbred mice population (Tarang 2018). The locus D8Mit14 had 6 genotypes (160/160, 160/150, 160/144, 150/150, 150/144 and 144/144) with genotype 160/144 having minimum genotype frequency 0.020 whereas genotype 150/150 having maximum genotypic frequency of 0.430. Similar to the present study the genotype 150/150 had the highest frequency in both the populations studied with the value of 0.420 in F_0 population and 0.433 in the F_1 inbred mice population (Tarang 2018).

The locus D9Mit27 had 6 genotypes (190/190, 190/178, 190/158, 178/178, 178/158, 158/158) with genotype 190/158 having minimum genotype frequency 0.010 whereas genotype 178/178 having maximum genotypic frequency of 0.540. While the genotype 190/158 had the highest frequency in the foundation population studied with genotypic frequency of 0.240, whereas similar to our current study for 178/178 in the F_1 inbred mice population two genotypes 178/178 and 158/158 were having the highest frequency (0.117 each) (Tarang 2018). The D10Mit180 locus showed 6 genotypes (224/224, 224/158, 224/138, 158/158, 158/138 and 138/138). Genotype 224/138 having minimum genotype frequency 0.110 whereas genotype 158/158 having maximum genotypic frequency of 0.260. Tarang (2018) however, reported that genotypes (224/138 and 158/138) had highest frequency in the foundation population with the value of 0.220 each while genotypes (224/224 and 158/158) had the highest frequency in the F_1 inbred mice population with the value of 0.217 each. The locus D11Mit167 had 6 genotypes (144/144, 144/128, 144/122, 128/128, 128/122 and 122/122) with genotype 128/122 having minimum genotype frequency 0.060 whereas genotype 128/128 having maximum genotypic frequency of 0.430. The genotype 122/122 was observed with highest frequency (0.260) in the foundation population whereas genotype 128/128 had highest frequency (0.500) in the F_1 inbred mice population (Tarang 2018).

Details of numbers of alleles, PIC, heterozygosity, allelic diversity and HWE statistics of all studied microsatellite loci in F_2 population are shown in Table 3. The range of PIC value (from 0.414 to 0.689) for various microsatellite loci revealed that population under investigation was of high diversity maintaining a multiple allele. While Purohit *et al.* (2015) reported PIC value as 0.395 (mean) for 14 different microsatellite markers. The PIC values of the loci ranged between 0.477–0.682 for foundation whereas ranged between 0.419–0.635 for F_1 inbred population (Tarang 2018). The maximum observed heterozygosity (H_o) was found in D1Mit15 as 0.660 and minimum in D5Mit18 as 0.080 with mean of 0.269. However, Purohit *et al.* (2015) reported that H_o was measured maximum in the marker D17Mit124 as 0.457 and minimum 0.528 in D17Mit62 with mean of 0.179 for 14 microsatellite loci. Likewise, 19% variation between FVB/NJ and C58/J and 21% between

Table 3. Details of No. of alleles, PIC, heterozygosity, allelic diversity and HWE statistics of all studied microsatellite loci in F₂ population

Locus	N	No of alleles	PIC	Heterozygosity	Allelic diversity	HWE χ^2
D1Mit15	100	4	0.665	0.660	0.718	0.0002
D2Mit51	100	4	0.689	0.470	0.737	<.0001
D2Mit61	100	3	0.568	0.310	0.642	<.0001
D3Mit15	100	3	0.414	0.110	0.458	<.0001
D3Mit55	100	4	0.646	0.140	0.698	<.0001
D5Mit18	100	4	0.598	0.080	0.666	<.0001
D7Mit323	100	4	0.667	0.240	0.719	<.0001
D8Mit14	100	3	0.534	0.190	0.608	<.0001
D9Mit27	100	3	0.462	0.130	0.538	<.0001
D10Mit180	100	3	0.585	0.390	0.659	<.0001
D11Mit167	100	3	0.534	0.240	0.608	<.0001

Table 4: Population genetic parameters for each microsatellite marker for F₂ inbred population

Locus	No. of alleles		Heterozygosity		F _{IS}
	Observed (Na)	Effective (Ne)	Observed (Ho)	Expected (He)	
D1Mit15	4	3.555	0.660	0.7224	0.0817
D2Mit51	4	3.814	0.470	0.7416	0.3630
D2Mit61	3	2.794	0.310	0.6454	0.5173
D3Mit15	3	1.847	0.110	0.4612	0.7603
D3Mit55	4	3.320	0.140	0.7024	0.7997
D5Mit18	4	2.994	0.080	0.6693	0.8799
D7Mit323	4	3.567	0.240	0.7233	0.6665
D8Mit14	3	2.551	0.190	0.6111	0.6875
D9Mit27	3	2.164	0.130	0.5407	0.7583
D10Mit180	3	2.934	0.390	0.6625	0.4084
D11Mit167	3	2.544	0.240	0.6117	0.6057
Mean	3.454	2.918	0.269	0.6447	0.592

*N_a, observed no. of alleles and N_e, effective no. of alleles.

FVB/NJ and I/LnJ strain of mice were observed by using 419 microsatellite loci (Voyer and Hunter, 1998). Basta *et al.* (2004) reported that FVB/N strain varied from the C34/HCNMTV strain by 67% and from C57BL/6N strain by 78%, using 27 microsatellites. Four strains (A1, T2, N2, and N4) had different genotypes at 13 out of the 30 microsatellite loci (Zhang *et al.* 2007). Tarang (2018) however, reported that Ho mean was 0.460 and 0.390 in foundation population and in F₁ inbred population, respectively.

Details of various population genetic parameters of each microsatellite marker in F₂ inbred population are given in Table 4. In current study the F_{IS} estimates ranged from 0.081 (D1Mit15) to 0.879 (D5Mit18). The highest within-population fixation index was observed for the D5Mit18 (0.879) microsatellites. The mean value of inbreeding coefficient based on marker data was positive and estimated as 0.592, indicating that parents were more related than expected under random mating. The results were in agreement with Shang *et al.* (2009), who observed that F_{st} per locus varied from 0.013 (D2Mit30) to 0.569 (D7Mit281) and the average F_{st} of all loci was 0.143. The rate of inbreeding, in minimize the rate of inbreeding (MRI)

complies 0.5% while in to conserve the phenotype of the base population (BPC) which was directed towards the maintenance of the phenotypic of the base population, the gain of inbreeding rate was 1.4% using microsatellite (Brockmann and Langhammer 1998). There was an increase of only 5.7% inbreeding in the F₁ inbred population in comparison to F₀ population based on 10 microsatellites (Tarang 2018).

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