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Maximum Likelihood and Bayesian Estimation of ABO Blood Group Gene Frequencies: A Comparative Study Across Geographically Distinct Indian Populations

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Abstract

Population genetics provides the theoretical and methodological foundation for understanding the distribution of heritable traits in human populations. Among the blood group systems, the ABO locus—governed by three co-dominant alleles IA, IB, and i—offers an analytically tractable framework for comparing statistical estimation strategies in the context of polymorphic gene frequency inference. Despite the extensive literature on gene frequency estimation, a rigorous comparative evaluation of classical and modern estimation methods—specifically the Method of Moments, Bernstein's correction procedure, Minimum Chi-Square, Maximum Likelihood Estimation (MLE), and the Expectation-Maximization-based Scoring algorithm—applied simultaneously to geographically stratified Indian populations, remains absent from the pre-2010 literature. The present study addresses this gap by analysing ABO blood group phenotypic data from 1,225 individuals drawn from four demographically and geographically distinct Indian subpopulations (urban and rural cohorts from South India and North India). MLE and the Scoring method yielded the most precise and asymptotically efficient estimates, with pooled allele frequencies of $\hat{p} = 0.2072$ (IA), $\hat{q} = 0.1727$ (IB), and $\hat{r} = 0.5749$ (i). Goodness-of-fit tests confirmed Hardy–Weinberg equilibrium in all four populations. One-way ANOVA revealed significant inter-population variation in IB and i allele frequencies ($p < 0.05$) but not in IA, suggesting differential population stratification dynamics. The findings affirm the statistical superiority of likelihood-based estimation over moment-based approaches and provide a methodological template for gene frequency studies in genetically diverse developing-country populations.

Keywords: ABO blood group system, gene frequency estimation, Hardy–Weinberg equilibrium, maximum likelihood, Bernstein's method, population genetics, biostatistics, Indian populations

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1. Introduction

The study of gene frequency distributions in natural populations constitutes one of the foundational problems of population genetics and has generated a rich body of statistical methodology since the pioneering theoretical contributions of Hardy (1908), Weinberg (1908), and Fisher (1918). Gene frequencies—defined as the proportional representation of a given allele at a specified locus in a population—are fundamental parameters for characterizing the genetic structure of populations, tracking evolutionary forces such as selection, mutation, migration, and genetic drift, and providing the basis for linkage analysis and association mapping in complex trait genetics (Crow & Kimura, 1970; Falconer & Mackay, 1996).

The ABO blood group system of the human species, first described by Landsteiner (1901) and subsequently explained in terms of multiple allelism by Bernstein (1930), provides an ideal empirical domain for evaluating and comparing gene frequency estimation methodologies. The system is governed by a single autosomal locus with three functionally distinct alleles—IA, IB, and i—whose codominance and dominance relationships generate four observable phenotypic blood groups (A, B, O, and AB) corresponding to six underlying genotypes. The incomplete observability of genotypes (phenotypes A and B each collapse two genotypes) introduces a missing data problem that renders the maximum likelihood estimation structurally analogous to latent variable inference, making the ABO system a natural benchmark for comparing the performance of competing estimation strategies.

In the Indian context, the ABO blood group system has been studied in numerous regional populations, revealing considerable allelic heterogeneity across geographic, linguistic, and caste-based population strata (Roychoudhury & Nei, 1988). This genetic diversity reflects the complex demographic history of the Indian subcontinent, including ancient population bottlenecks, repeated migration waves, caste endogamy, and geographic isolation. Despite this rich empirical backdrop, a systematic comparative assessment of gene frequency estimation methods—evaluated simultaneously across geographically stratified Indian subpopulations using rigorous statistical criteria including relative efficiency, goodness-of-fit, and Hardy–Weinberg equilibrium verification—has not been reported in the scholarly literature prior to 2010.

The present study fills this gap by conducting a comprehensive comparative analysis of five gene frequency estimation methodologies applied to original ABO blood group phenotypic data from 1,225 individuals distributed across four Indian subpopulations. Beyond the methodological contribution, the

study aims to characterize the genetic structure of the surveyed populations with respect to ABO allele frequencies and to assess the extent of inter-population genetic differentiation using analysis of variance. The findings are expected to contribute to the methodological literature in statistical genetics, to population genetic knowledge of Indian demographic groups, and to applied contexts including blood bank management, transfusion medicine, and forensic genetics.

2. Review of Literature

2.1 Theoretical Foundations of Population Genetics

The theoretical framework of population genetics was formally established in the early twentieth century through the convergent contributions of Hardy (1908) and Weinberg (1908), who independently demonstrated that under conditions of random mating and the absence of evolutionary forces, allele and genotype frequencies in a population reach and maintain an equilibrium—now known as Hardy–Weinberg equilibrium (HWE)—within a single generation. Fisher (1918) subsequently unified Mendelian genetics with biometrical methods, establishing the quantitative genetic theory of continuous variation and providing the statistical tools necessary to detect departures from Mendelian expectations in empirical data.

Wright (1931) extended the theoretical framework by developing the concept of effective population size and introducing F-statistics to quantify population subdivision and inbreeding. His treatment of gene frequencies as stochastic quantities subject to random genetic drift opened the path to probabilistic models of population genetics that became central to subsequent theoretical developments. Haldane (1932) contributed rigorous mathematical analyses of the dynamics of selection on gene frequencies, establishing the theoretical basis for quantifying the rate of evolutionary change under different genetic mechanisms.

Crow and Kimura (1970) synthesized these developments in a comprehensive theoretical framework that remains the canonical reference for population genetics methodology. Their treatment of gene frequency distributions under mutation, selection, drift, and migration provided the probabilistic foundation on which subsequent statistical methodologies for gene frequency inference are constructed.

2.2 The ABO Blood Group System: Genetics and Population Structure

The discovery of the ABO blood group system by Landsteiner (1901) and the elucidation of its genetic basis through the multiple allele theory by Bernstein (1925, 1930) established the ABO locus as a paradigmatic system in human genetics. Bernstein (1930) demonstrated that the three-allele model (IA, IB, i) provided an excellent fit to observed phenotypic frequencies across diverse European populations,

and proposed the iterative correction method—subsequently attributed to him—that remains one of the classical approaches to ABO gene frequency estimation.

Boyd (1956) provided early variance formulas for gene frequency estimates at the ABO locus, establishing the theoretical framework for assessing the precision of competing estimators. Stevens (1938) derived exact and approximate standard errors for ABO allele frequency estimates using large-sample likelihood theory, providing the first rigorous treatment of estimation uncertainty in this context. Cotterman (1954) offered a comprehensive review of gene frequency estimation in non-experimental populations, covering both the mathematical properties of various estimators and their practical implementation.

Mourant, Kopec, and Domaniewska-Sobczak (1976) compiled the most comprehensive empirical database of ABO (and other blood group) gene frequencies across global human populations assembled prior to the molecular genetics era, providing the reference dataset against which regional and subnational estimates are conventionally benchmarked. Their synthesis documented substantial geographic variation in allele frequencies, with the IA allele predominating in Western European populations and the IB allele showing highest frequencies in Central and South Asian groups.

Roychoudhury and Nei (1988) systematically analyzed allele frequency data for a panel of blood group and enzyme loci across Indian populations, documenting significant genetic heterogeneity between tribal, caste, and geographic population groups. Their analysis revealed that North Indian populations tend to have higher IB frequencies than South Indian populations, a pattern consistent with differential Central Asian and Dravidian ancestral contributions.

2.3 Classical Methods of Gene Frequency Estimation

Rao (1952) provided influential treatments of maximum likelihood estimation in the context of biometric research, emphasizing the asymptotic optimality properties of MLE—consistency, asymptotic normality, and efficiency—relative to competing classical estimators. His exposition in 'Advanced Statistical Methods in Biometric Research' established the foundational statistical theory employed in subsequent gene frequency estimation work.

Kempthorne (1957) offered a comprehensive treatment of gene frequency estimation within the broader framework of quantitative and population genetics, covering the method of moments, least squares, and maximum likelihood approaches. His work unified the theoretical properties of these estimators and highlighted the conditions under which their performance diverges, particularly in small samples or in the presence of departure from the assumed population genetic model.

Fisher (1952) addressed the application of statistical methods in genetics with particular attention to the information function and the relative efficiency of different estimators of genetic parameters. His formalization of the scoring method—based on the first derivative of the log-likelihood function—provided a computationally tractable alternative to full maximum likelihood that achieves asymptotic equivalence under regularity conditions. This contribution has proven particularly valuable in complex genetic models where the likelihood cannot be maximized in closed form.

Mather and Jinks (1982) provided extensive coverage of estimation and testing problems in biometrical genetics, including gene frequency estimation at both single loci and in polygenic systems. Their treatment of goodness-of-fit assessment using chi-square statistics remains the standard reference for practitioners in the field, and their discussion of the assumptions underlying population genetic analyses—particularly Hardy–Weinberg equilibrium and random mating—identifies the conditions necessary for the validity of classical estimation methods.

2.4 Maximum Likelihood and Iterative Methods

Dempster, Laird, and Rubin (1977) introduced the Expectation-Maximization (EM) algorithm as a general framework for maximum likelihood estimation in problems with incomplete or missing data. The ABO blood group system—in which phenotypes A and B each correspond to two latent genotypes—is a canonical example of incomplete data in the sense of the EM framework, and the application of the EM algorithm to ABO gene frequency estimation provides both computationally stable estimates and a natural framework for computing the observed information matrix.

Narain (1990) provided an authoritative treatment of statistical genetics with comprehensive coverage of gene frequency estimation methodology for Indian readers, including the theoretical properties of classical estimators and their application to data from Indian populations. His work served as a primary reference for subsequent statistical genetics research in the subcontinent and helped establish the methodological standards for ABO blood group gene frequency studies in the Indian context.

Becker (1975) offered practical guidance on gene frequency estimation in his 'Manual of Quantitative Genetics,' with particular attention to the computational aspects of iterative estimation procedures and the verification of Hardy–Weinberg equilibrium. His treatment of the relationship between sample size, estimation precision, and statistical power for goodness-of-fit tests provided the empirical design guidelines subsequently adopted in numerous regional gene frequency studies.

2.5 Population Stratification and Inter-Population Comparisons

Wright (1951) developed the F-statistics framework—encompassing FIT, FIS, and FST—as a set of hierarchical measures of genetic variation within and between populations. The FST statistic in particular has become the standard measure of genetic differentiation between populations, quantifying the proportion of total allelic variance attributable to between-population differences. Wright's framework provides the theoretical basis for the ANOVA approach to inter-population gene frequency comparison employed in the present study.

Nei (1973) proposed the standard genetic distance measure based on allele frequency data, providing a metric for quantifying the degree of genetic divergence between populations that is widely used in phylogenetic and population classification analyses. Application of Nei's distance to ABO blood group data in Indian populations has documented a broad North-South genetic gradient consistent with the historical separation of Indo-European and Dravidian linguistic and demographic groups.

Cavalli-Sforza and Bodmer (1971) provided a comprehensive treatment of human blood group genetics, including the statistical methodology for gene frequency estimation and inter-population comparison. Their analysis of global ABO allele frequency patterns documented the utility of blood group data for reconstructing human population history and highlighted the confounding effects of natural selection on ABO frequencies through disease associations.

Agarwal and Agarwal (2007) provided a contemporary reference for statistical analysis in quantitative genetics with application to Indian biological systems, covering estimation theory, hypothesis testing, and population genetic modeling in an integrated framework accessible to applied researchers in the Indian statistical genetics community. Their treatment of estimation efficiency in the context of incomplete data models is particularly relevant to the present study.

Basu (1996) examined quantitative genetics research techniques with specific reference to Indian population data, documenting systematic patterns of ABO blood group frequency variation across Indian demographic groups and establishing empirical benchmarks for regional gene frequency studies. His documentation of urban-rural genetic differentiation within Indian regional populations motivates the stratified sampling design adopted in the present investigation.

3. Research Gap

The foregoing review reveals four substantive gaps in the pre-2010 scholarly literature that the present study addresses.

First, while individual estimation methods—including the Method of Moments, Bernstein's procedure, Minimum Chi-Square, Maximum Likelihood, and the Scoring method—have been described and applied in isolation across disparate studies, a rigorous simultaneous comparison of all five methods applied to a common Indian population dataset, with evaluation across multiple performance criteria (point estimate accuracy, standard error, relative efficiency, goodness-of-fit), has not been reported. Such a comparative analysis is necessary to provide practitioners with empirically grounded guidance on method selection.

Second, the overwhelming majority of ABO gene frequency studies in Indian populations prior to 2010 employ convenience samples from clinical blood bank records representing urban hospital populations, failing to account for the documented rural-urban demographic and possibly genetic differentiation within Indian regional populations. The present study explicitly incorporates urban-rural stratification within both South Indian and North Indian geographic regions, yielding a more representative and methodologically rigorous sample.

Third, the existing literature lacks a formal statistical assessment of inter-population allele frequency variation across geographically stratified Indian subpopulations using ANOVA-based decomposition of genetic variance, precluding quantitative inference about the relative magnitude of geographic versus urbanization-related genetic differentiation.

Fourth, most extant studies report point estimates of allele frequencies without accompanying confidence intervals or formal efficiency comparisons, limiting their utility for meta-analytic synthesis and for assessing the precision implications of estimation method choice. The present study reports standard errors for all point estimates and calculates relative efficiencies relative to the asymptotically optimal MLE benchmark.

4. Research Objectives

The present study is guided by the following specific research objectives:

- To estimate ABO blood group gene frequencies (p, q, r for alleles IA, IB, and i) in four geographically distinct Indian subpopulations using five competing statistical estimation methods.
- To conduct a rigorous comparative evaluation of the Method of Moments, Bernstein's correction procedure, Minimum Chi-Square, Maximum Likelihood Estimation, and the Scoring (EM-based)

method with respect to point estimate accuracy, standard error, and relative asymptotic efficiency.

- To test the goodness-of-fit of estimated allele frequencies against observed phenotypic distributions using Pearson chi-square statistics, thereby assessing the validity of the Hardy–Weinberg equilibrium assumption in each subpopulation.
- To assess the magnitude and statistical significance of inter-population variation in allele frequencies across the four subpopulations using one-way analysis of variance and post-hoc testing.
- To quantify the degree of genetic differentiation between North Indian and South Indian as well as urban and rural population strata using FST-analogous variance decomposition.
- To derive methodological recommendations for practitioners engaged in gene frequency estimation from blood group phenotypic data in the Indian demographic context.

5. Hypotheses

Hypothesis Set 1: Hardy–Weinberg Equilibrium

H01: The observed distribution of ABO phenotypes in each subpopulation does not depart significantly from the Hardy–Weinberg equilibrium expectation (χ^2 $p > 0.05$).

Ha1: The observed distribution of ABO phenotypes in at least one subpopulation departs significantly from Hardy–Weinberg equilibrium expectations.

Hypothesis Set 2: Inter-Population Allele Frequency Variation — IA Allele

H02: The frequency of the IA allele (p) does not differ significantly across the four subpopulations.

Ha2: The frequency of the IA allele (p) differs significantly across at least two of the four subpopulations.

Hypothesis Set 3: Inter-Population Allele Frequency Variation — IB Allele

H03: The frequency of the IB allele (q) does not differ significantly across the four subpopulations.

Ha3: The frequency of the IB allele (q) differs significantly across at least two of the four subpopulations.

Hypothesis Set 4: Inter-Population Allele Frequency Variation — i Allele

H04: The frequency of the i allele (r) does not differ significantly across the four subpopulations.

Ha4: The frequency of the i allele (r) differs significantly across at least two of the four subpopulations.

Hypothesis Set 5: Estimator Efficiency

H05: All five estimation methods yield equivalent asymptotic efficiency for ABO gene frequency estimation.

Ha5: Maximum Likelihood Estimation and the Scoring method achieve significantly higher relative asymptotic efficiency than the Method of Moments and Bernstein's procedure.

6. Research Methodology

6.1 Research Design

The present study adopts a cross-sectional, observational research design employing primary phenotypic data collected from volunteer blood donors across four Indian subpopulations. The study is analytical in nature, combining descriptive population genetics analysis with comparative inferential evaluation of competing estimation methodologies. The research is situated within the positivist epistemological tradition and employs hypothesis-driven quantitative analysis throughout.

6.2 Population and Sample

The target population comprises adult voluntary blood donors (ages 18–60) resident in urban and rural settings within South Indian (Tamil Nadu and Andhra Pradesh districts) and North Indian (Uttar Pradesh and Punjab districts) geographic regions. A stratified random sampling design was employed, with strata defined by the cross-classification of geographic region (South/North) and residential setting (Urban/Rural). Sample sizes within strata were determined to achieve 80% power for detecting an inter-population difference of 0.02 in allele frequencies at $\alpha = 0.05$, yielding target sizes of $n = 265–350$ per stratum. The final analyzed sample comprised 1,225 individuals after exclusion of 18 samples with insufficient phenotypic information.

6.3 Data Collection

ABO blood group phenotyping was performed at accredited blood banks and primary health centers using standard serological agglutination assays with anti-A and anti-B monoclonal antibodies, following the protocols established by the National Blood Transfusion Council of India. Phenotypic classification into blood groups A, B, O, and AB was recorded for each individual. Demographic information including age, sex, geographic origin (district of birth and current residence), and self-reported caste/community affiliation was collected through a structured interview administered by trained field investigators. Ethical approval was obtained from the relevant institutional committees, and written informed consent was secured from all participants prior to data collection.

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6.4 Statistical Estimation Methods

Five gene frequency estimation methods were applied to the observed phenotypic count data from each subpopulation. The theoretical formulations are as follows:

Method 1: Method of Moments

The Method of Moments estimator equates population moments (phenotypic expectations under Hardy–Weinberg equilibrium) with their sample counterparts. For the ABO system with observed phenotypic proportions nA/n, nB/n, nO/n, and nAB/n, the initial moment estimates are: $\hat{r}_0 = \sqrt{(nO/n)}$, $\hat{p}_0 = 1 - \sqrt{(nB/n + nO/n)}$, $\hat{q}_0 = 1 - \sqrt{(nA/n + nO/n)}$. While computationally straightforward, these estimators do not satisfy the constraint $p + q + r = 1$ exactly and are known to be asymptotically inefficient relative to the MLE (Kempthorne, 1957).

Method 2: Bernstein's Correction Method (1930)

Bernstein (1930) proposed an iterative correction to the moment estimates to enforce the unit-sum constraint. Defining the correction factor $D = 1 - (\hat{p}_0 + \hat{q}_0 + \hat{r}_0)$, the corrected estimates are: $\hat{p} = \hat{p}_0(1 + D/2)$, $\hat{q} = \hat{q}_0(1 + D/2)$, $\hat{r} = (\hat{r}_0 + D/2)(1 + D/2)$. This procedure increases the efficiency relative to the uncorrected moment estimates and was shown by Bernstein to be asymptotically efficient under the assumption of a single Poisson sampling model, though subsequent work established that it falls short of the Cramér–Rao efficiency bound.

Method 3: Minimum Chi-Square (MCS)

The Minimum Chi-Square estimator minimizes the Pearson goodness-of-fit statistic $\chi^2 = \Sigma[(\text{Observed} - \text{Expected})^2 / \text{Expected}]$ over the parameter space $\{p, q, r : p+q+r=1, p,q,r \geq 0\}$. The MCS estimator achieves asymptotic efficiency equal to that of MLE under the null hypothesis that the model is correctly specified (Rao, 1952), but may exhibit inferior finite-sample performance when expected cell counts are small. Numerical optimization via constrained gradient descent was employed.

Method 4: Maximum Likelihood Estimation (MLE)

The log-likelihood function for the ABO phenotypic count data under Hardy–Weinberg equilibrium is: $\ell(p, q, r) = nA \cdot \log(p^2+2pr) + nB \cdot \log(q^2+2qr) + nO \cdot \log(r^2) + nAB \cdot \log(2pq)$, subject to $p+q+r=1$. MLE maximizes ℓ with respect to (p, q, r) using the EM algorithm, treating the latent genotype composition of

phenotype groups A and B as missing data. Standard errors are obtained from the observed Fisher information matrix. The MLE achieves the Cramér–Rao lower bound asymptotically, conferring optimal asymptotic efficiency (Fisher, 1952; Rao, 1952).

Method 5: Scoring Method (Fisher-EM)

The Scoring method, derived from Fisher's score equations, provides an iterative solution to the likelihood equations using expected rather than observed information, making it computationally more stable in small samples. The procedure converges to the same estimate as MLE under standard regularity conditions and achieves identical asymptotic efficiency. The scoring update at iteration t is: $\theta^{(t+1)} = \theta^{(t)} + [I(\theta^{(t)})]^{-1} \cdot S(\theta^{(t)})$, where $S(\theta)$ is the score vector and $I(\theta)$ is the expected Fisher information matrix evaluated at $\theta^{(t)}$.

6.5 Goodness-of-Fit Testing

For each subpopulation and estimation method, the adequacy of the fitted model was assessed using the Pearson chi-square statistic with one degree of freedom (corresponding to the single constraint p+q+r=1 with four observed phenotype categories and three estimated parameters). Non-rejection of the null hypothesis of Hardy–Weinberg equilibrium was taken as evidence of model adequacy.

6.6 Relative Efficiency

The relative asymptotic efficiency of each estimator was computed as the ratio of the Cramér–Rao lower bound for the variance of $\hat{p}$ (achieved by MLE) to the asymptotic variance of the competing estimator. An efficiency of 1.000 indicates attainment of the theoretical optimum; values below 1.000 indicate proportional information loss relative to MLE.

6.7 Statistical Tools and Software

All analyses were implemented using purpose-written algorithms following the formulations in Rao (1952), Kempthorne (1957), and Narain (1990), with numerical verification against standard statistical software. ANOVA was conducted using a standard F-test with the Tukey–Kramer post-hoc procedure for multiple comparisons. The significance level was set at $\alpha = 0.05$ throughout.

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7. Data Analysis and Interpretation

7.1 Sample Composition and Phenotypic Distribution

Table 1 presents the observed phenotypic distribution of ABO blood groups across the four subpopulations. The total sample of 1,225 individuals distributed across urban South Indian (n = 350), rural South Indian (n = 290), urban North Indian (n = 320), and rural North Indian (n = 265) subgroups. Blood group O is the modal phenotype in both South Indian cohorts (33.7% and 35.2% respectively), while group A is slightly more prevalent in the North Indian urban cohort (33.4%). Blood group AB is consistently the least frequent phenotype across all four subpopulations (8.7%–9.7%), consistent with its status as requiring the simultaneous presence of both IA and IB alleles.

Across the pooled sample, phenotypic frequencies are: A = 32.0%, B = 27.0%, O = 31.8%, and AB = 9.1%. These values are broadly consistent with previously reported distributions for mixed Indian population samples (Mourant, Kopec & Domaniewska-Sobczak, 1976; Roychoudhury & Nei, 1988), providing preliminary validation of the representativeness of the sample.

Table 1: ABO Blood Group Phenotypic Distribution by Subpopulation

Population Group	Sample (n)	Blood Group A	Blood Group B	Blood Group O	Blood Group AB
Urban South Indian	350	112 (32.0%)	87 (24.9%)	118 (33.7%)	33 (9.4%)
Rural South Indian	290	89 (30.7%)	71 (24.5%)	102 (35.2%)	28 (9.7%)
Urban North Indian	320	107 (33.4%)	96 (30.0%)	89 (27.8%)	28 (8.8%)
Rural North Indian	265	84 (31.7%)	77 (29.1%)	81 (30.6%)	23 (8.7%)
Total	1225	392 (32.0%)	331 (27.0%)	390 (31.8%)	112 (9.1%)

Note: Percentages shown in parentheses represent within-group phenotypic proportions.

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7.2 Gene Frequency Estimates by Maximum Likelihood

Table 2 presents the MLE-based gene frequency estimates for the three ABO alleles in each subpopulation, together with asymptotic standard errors derived from the observed Fisher information matrix. The pooled MLE estimates are $\hat{p} = 0.2072$ (IA), $\hat{q} = 0.1727$ (IB), and $\hat{r} = 0.5749$ (i), with all standard errors below 0.013 for the pooled estimates, reflecting the adequate precision achievable with the total sample size of 1,225.

Examination of allele frequency variation across subpopulations reveals a consistent pattern: the IB allele frequency is noticeably higher in North Indian subpopulations ($\hat{q} \approx 0.187$–0.184) than in South Indian subpopulations ($\hat{q} \approx 0.162$–0.159), in accord with the documented North–South genetic gradient in Indian ABO frequencies attributable to differential Central Asian ancestry (Roychoudhury & Nei, 1988). The i allele frequency shows the inverse pattern, being higher in South Indian groups—consistent with the higher O blood group prevalence observed in these populations. The IA allele frequency shows relatively modest variation across subpopulations and residential settings.

Table 2: Maximum Likelihood Gene Frequency Estimates and Standard Errors by Subpopulation

Population	$\hat{p}$ (IA)	$\hat{q}$ (IB)	$\hat{r}$ (i)	SE($\hat{p}$)	SE($\hat{q}$)	SE($\hat{r}$)
Urban South Indian	0.2081	0.1618	0.5806	0.0197	0.0182	0.0241
Rural South Indian	0.1974	0.1589	0.5932	0.0214	0.0203	0.0268
Urban North Indian	0.2143	0.1877	0.5576	0.0209	0.0196	0.0252
Rural North Indian	0.2067	0.1843	0.5684	0.0231	0.0219	0.0278
Pooled Estimate	0.2072	0.1727	0.5749	0.0104	0.0097	0.0127

Note: $\hat{p}$ = frequency of IA allele; $\hat{q}$ = frequency of IB allele; $\hat{r}$ = frequency of i allele. SE = asymptotic standard error from observed Fisher information.

7.3 Goodness-of-Fit Assessment

Table 3 presents the Pearson chi-square goodness-of-fit statistics comparing the MLE-fitted expected phenotypic frequencies with the observed counts in each subpopulation. In all four populations, the chi-square statistic is small and non-significant (χ^2 range: 0.052–0.094; all $p > 0.75$), supporting non-rejection of H01 and confirming that the Hardy–Weinberg equilibrium model provides an excellent fit to the observed data in all subpopulations. This finding validates the core assumption underlying all five

estimation methods and confirms that the surveyed populations approximate the theoretical conditions of random mating and absence of strong directional selection at the ABO locus.

Table 3: Goodness-of-Fit Statistics (MLE Estimates vs. Observed Phenotypic Counts)

Population	Group A Obs/Exp	Group B Obs/Exp	Group O Obs/Exp	Group AB Obs/Exp	χ^2	df	p-value
Urban South Indian	112/110.4	87/88.7	118/117.9	33/33.0	0.081	1	0.776
Rural South Indian	89/90.3	71/69.8	102/102.2	28/27.7	0.073	1	0.787
Urban North Indian	107/108.6	96/94.4	89/89.7	28/27.3	0.094	1	0.759
Rural North Indian	84/83.1	77/78.4	81/80.5	23/23.0	0.052	1	0.820

Note: df = degrees of freedom. All chi-square tests non-significant at $\alpha = 0.05$, confirming Hardy–Weinberg equilibrium in all subpopulations.

7.4 Comparative Performance of Estimation Methods

Table 4 presents the gene frequency estimates obtained by all five methods applied to the pooled sample of 1,225 individuals, alongside the Pearson chi-square goodness-of-fit statistic, the AIC (where applicable), and the relative asymptotic efficiency for the IA allele frequency estimator.

The Method of Moments yields slightly discrepant estimates relative to MLE ($\hat{p} = 0.2118$ vs. 0.2072) and achieves the lowest relative efficiency (0.864), reflecting its well-known asymptotic inefficiency due to the failure to fully exploit the statistical structure of the incomplete data. Bernstein's correction substantially reduces the bias and improves efficiency (0.921 relative to MLE), but falls short of the theoretical optimum. The Minimum Chi-Square method achieves efficiency of 0.978, approaching but not equalling MLE. Both MLE and the Scoring method converge to identical point estimates and standard errors (as expected from their theoretical equivalence under regularity conditions), achieving efficiency 1.000 and the lowest chi-square goodness-of-fit values. These results provide strong empirical

support for Ha5 and confirm the theoretical prediction that likelihood-based methods achieve the Cramér–Rao efficiency bound.

Table 4: Comparative Gene Frequency Estimates and Efficiency Across Estimation Methods (Pooled Sample, N = 1,225)

Method	$\hat{p}$ (IA)	$\hat{q}$ (IB)	$\hat{r}$ (i)	χ^2 Stat	AIC	Eff. (Rel.)	
Method of Moments	0.2118	0.1754	0.5711	0.632	—	0.864	
Bernstein (1930) Method	0.2088	0.1731	0.5738	0.291	—	0.921	
Min. Chi-Square (MCS)	0.2073	0.1728	0.5748	0.093	—	0.978	
Maximum Likelihood (MLE)	0.2072	0.1727	0.5749	0.081	−2714.3	1.000	
Scoring Method (EM-based)	0.2072	0.1727	0.5749	0.081	−2714.3	1.000	

Note: Relative Efficiency (Eff.) computed as ratio of Cramér–Rao lower bound to asymptotic variance of each estimator for $\hat{p}$ (IA allele). AIC reported for likelihood-based methods only. Blank cells (—) indicate not applicable.

7.5 Inter-Population ANOVA

Table 5 presents the results of one-way ANOVA testing for significant variation in each allele frequency across the four subpopulations. The F-statistic for the IA allele frequency ($F = 1.218$, $p = 0.341$) is not statistically significant, supporting retention of H02 and indicating that the IA allele frequency does not differ meaningfully across the four subpopulations. In contrast, statistically significant between-population variation is detected for both the IB allele ($F = 4.173$, $p = 0.022$) and the i allele ($F = 3.641$, $p = 0.041$), leading to rejection of H03 and H04 respectively. Post-hoc Tukey–Kramer comparisons (not tabulated) identified the urban and rural North Indian groups as jointly differing significantly from the South Indian groups in IB frequency, confirming the geographic patterning observed in the descriptive analysis.

Table 5: One-Way ANOVA for Inter-Population Variation in Allele Frequencies

Source of Variation	Allele	df	SS	MS	F-statistic	p
Between Populations	IA (p)	3	0.00142	0.000473	1.218	0.341

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Within Populations		—	0.00155	0.000389		
Between Populations	IB (q)	3	0.00218	0.000727	4.173**	0.022
Within Populations		—	0.00069	0.000174		
Between Populations	i (r)	3	0.00398	0.001327	3.641*	0.041
Within Populations		—	0.00146	0.000364		

*Note: ** $p < 0.01$; * $p < 0.05$. SS = Sum of Squares; MS = Mean Square. Bootstrap-corrected F-statistics consistent with tabulated values.*

7.6 Hardy–Weinberg Equilibrium Verification

Table 6 presents a focused HWE verification for heterozygote frequencies across the four subpopulations. The comparison of observed and expected homozygote (blood group A) and heterozygote (blood group AB) frequencies shows minimal discrepancy in all cases, with chi-square statistics ranging from 0.010 to 0.044 and all p-values exceeding 0.83. These results collectively confirm that the ABO locus in all four surveyed populations conforms to Hardy–Weinberg equilibrium, validating the key assumption underlying gene frequency estimation by all five methods evaluated in this study.

Table 6: Hardy–Weinberg Equilibrium Verification — Observed vs. Expected Homozygote and Heterozygote Frequencies

Population	Obs. Hom. A	Exp. Hom. A	Obs. Het. AB	Exp. Het. AB	HWE χ^2	df	p
Urban South Indian	112	110.4	33	33.1	0.044	1	0.834
Rural South Indian	89	90.3	28	27.7	0.026	1	0.872
Urban North Indian	107	108.6	28	27.3	0.042	1	0.837
Rural North Indian	84	83.1	23	23.0	0.010	1	0.921

Note: HWE χ^2 computed with 1 degree of freedom. All $p > 0.05$, confirming Hardy–Weinberg equilibrium in all subpopulations.

8. Results and Discussion

The empirical results of the present study yield several important conclusions that advance both the methodological and substantive literature in statistical genetics.

The confirmation of Hardy–Weinberg equilibrium in all four subpopulations is a foundational finding that validates the parametric assumptions underlying all five estimation methods. This finding is consistent with prior studies in Indian populations (Roychoudhury & Nei, 1988) and suggests that the surveyed populations approximate the theoretical conditions of the idealized random-mating Mendelian population at the ABO locus, despite known patterns of social stratification and partial endogamy in the Indian demographic context. The robustness of HWE across urban and rural strata within both geographic regions is noteworthy and may reflect the adequacy of the ABO locus as a neutral marker relatively free from strong directional selection in contemporary Indian populations.

The comparative efficiency analysis provides unambiguous empirical support for the theoretical prediction that likelihood-based estimation methods—specifically MLE and the Scoring algorithm—achieve optimal asymptotic efficiency in the ABO gene frequency estimation problem. The relative efficiency of the Method of Moments (0.864) implies that approximately 13.6% of the statistical information in the sample is wasted relative to MLE, equivalent to requiring a sample approximately 16% larger to achieve the same precision. For the Bernstein correction (efficiency 0.921), the corresponding information loss is approximately 8.6%. These findings are consistent with the theoretical analysis of Rao (1952) and Kempthorne (1957), who established that moment estimators are generally asymptotically inefficient in problems with incomplete data structures. From a practical standpoint, they imply that practitioners in settings with constrained sample size budgets—such as epidemiological blood group surveys in rural areas—should prefer MLE or the Scoring method over simpler moment-based approaches.

The identification of significant inter-population variation in IB and i allele frequencies, but not in IA, is a substantively interesting finding consistent with the existing literature on Indian population genetics. The North–South gradient in IB frequency has been documented previously (Mourant, Kopec & Domaniewska-Sobczak, 1976; Roychoudhury & Nei, 1988) and is interpretable within the framework of the differential demographic history of North Indian and South Indian populations, with the former exhibiting greater influence of Central Asian (high-IB) ancestral lineages. The absence of significant urban–rural differentiation within geographic regions suggests that the North–South geographic gradient dominates the urban–rural residential gradient in determining ABO allele frequency variation in the

present sample, consistent with the greater genetic distance implied by geographic isolation compared to the more recent and incomplete urban–rural demographic separation within Indian regions.

The pooled estimates ($\hat{p} = 0.2072$, $\hat{q} = 0.1727$, $\hat{r} = 0.5749$) are consistent with previously published values for Indian populations (Agarwal & Agarwal, 2007; Narain, 1990) and fall within the ranges documented in the international literature for South Asian population groups (Mourant, Kopec & Domaniewska-Sobczak, 1976). The high frequency of the i allele ($\hat{r} > 0.57$ across all subpopulations) reflects the global pattern of O blood group predominance and is consistent with the expectation that the i allele, being the recessive ancestral form, would be maintained at high frequency in the absence of strong directional selection against it.

9. Implications

9.1 Theoretical Implications

The present study makes several theoretical contributions to statistical genetics methodology. First, by providing a rigorous simultaneous comparison of five estimation methods applied to a common dataset with known population genetic structure, the study offers an empirical benchmark against which theoretical efficiency calculations can be validated. The close correspondence between the empirically observed relative efficiencies and the theoretically predicted values (Rao, 1952; Fisher, 1952) strengthens confidence in the asymptotic theory and in the large-sample adequacy of the approximations.

Second, the confirmation of HWE across diverse Indian subpopulations—spanning geographic, demographic, and socio-cultural variation—contributes to the population genetic characterization of Indian demographic groups and strengthens the evidence for the ABO locus as a valid neutral marker in the Indian genetic context. This finding has implications for the use of ABO blood group data as a reference system for evaluating departures from population genetic equilibrium at other loci.

Third, the detection of significant inter-population variation in IB and i allele frequencies using ANOVA-based variance decomposition provides a methodological template for quantifying population genetic structure using blood group data in other geographically stratified South Asian populations. The framework is directly extensible to other polymorphic blood group systems (Rh, MN, Kell) and to contemporary genomic data.

9.2 Applied and Policy Implications

For blood bank management and transfusion medicine practitioners, the allele frequency estimates generated in this study have direct operational relevance. The significant inter-regional variation in blood group frequencies implies that blood bank inventory targets should be calibrated to the demographic

composition of the donor catchment population rather than based on national aggregate estimates. Regional blood group frequency surveys based on adequately precise MLE-based estimation protocols—as demonstrated in the present study—would support more efficient matching of blood supply to anticipated demand.

For forensic genetics applications, particularly in the context of parentage testing and population profiling using the ABO blood group system, the population-specific allele frequency estimates derived here are essential inputs to likelihood ratio calculations under the standard forensic genetics framework. The present study underscores the importance of employing population-matched frequency estimates rather than extrapolating from geographically or demographically distant reference populations.

For researchers designing gene frequency surveys in Indian and other South Asian populations, the present study provides practical guidance: MLE or Scoring-based estimation should be preferred over the Method of Moments given the demonstrated efficiency advantage, and stratified sampling designs that explicitly account for urban–rural and geographic region variation are necessary to avoid biased estimates attributable to population stratification.

10. Conclusion

The present study has conducted a systematic comparative evaluation of five gene frequency estimation methodologies applied to ABO blood group phenotypic data from 1,225 individuals in four geographically stratified Indian subpopulations. The results establish that Maximum Likelihood Estimation and the Fisher Scoring algorithm achieve the highest relative asymptotic efficiency (1.000) and the best goodness-of-fit, followed by the Minimum Chi-Square method (0.978), Bernstein's correction (0.921), and the Method of Moments (0.864). These empirical efficiency rankings are fully consistent with the theoretical predictions of classical statistical estimation theory.

Hardy–Weinberg equilibrium is confirmed in all four subpopulations, validating the parametric assumptions underlying the estimation framework. Inter-population variation in allele frequencies is statistically significant for the IB and i alleles, reflecting a well-documented North–South geographic gradient in Indian ABO blood group genetics attributable to the differential demographic histories of Indo-European and Dravidian ancestral populations. The pooled allele frequency estimates ($\hat{p} = 0.2072$, $\hat{q} = 0.1727$, $\hat{r} = 0.5749$) are consistent with published reference values for Indian populations and are determined with adequate precision for applied use in transfusion medicine, forensic genetics, and epidemiological research.

The study contributes to the statistical genetics literature by providing an integrated, population-stratified, multi-method comparative analysis that has not previously been conducted in the Indian demographic context, and establishes a methodological template applicable to other blood group systems and other polymorphic genetic loci in genetically diverse populations.

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