

Experimental Design and Data Analysis for Biologists

Gerry P. Quinn Michael J. Keough

CAMBRIDGE

Contents

P	reface	page xv
l	Introduction	1
	1.1 Scientific method	1
	1.1.1 Pattern description	2
	1.1.2 Models	2
	1.1.3 Hypotheses and tests	3
	1.1.4 Alternatives to falsification	4
	1.1.5 Role of statistical analysis	5
	1.2 Experiments and other tests	5
	1.3 Data, observations and variables	7
	1.4 Probability	7
	1.5 Probability distributions	9
	1.5.1 Distributions for variables	10
	1.5.2 Distributions for statistics	12
2	Estimation	14
	2.1 Samples and populations	14
	2.2 Common parameters and statistics	15
	2.2.1 Center (location) of distribution	15
	2.2.2 Spread or variability	16
	2.3 Standard errors and confidence intervals for the mean	17
	2.3.1 Normal distributions and the Central Limit Theorem	17
	2.3.2 Standard error of the sample mean	18
	2.3.3 Confidence intervals for population mean	19
	2.3.4 Interpretation of confidence intervals for population mean	ı 20
	2.3.5 Standard errors for other statistics	20
	2.4 Methods for estimating parameters	23
	2.4.1 Maximum likelihood (ML)	23
	2.4.2 Ordinary least squares (OLS)	24
	2.4.3 ML vs OLS estimation	25
	2.5 Resampling methods for estimation	25
	2.5.1 Bootstrap	25
	2.5.2 Jackknife	26
	2.6 Bayesian inference – estimation	27
	2.6.1 Bayesian estimation	27
	2.6.2 Prior knowledge and probability	28
	2.6.3 Likelihood function	28
	2.6.4 Posterior probability	28
	2.6.5 Examples	29
	2.6.6 Other comments	29

3	İΗ	ypothesis testing	32
	3.1	Statistical hypothesis testing	32
		3.1.1 Classical statistical hypothesis testing	32
		3.1.2 Associated probability and Type I error	34
		3.1.3 Hypothesis tests for a single population	35
		3.1.4 One- and two-tailed tests	37
		3.1.5 Hypotheses for two populations	37
		3.1.6 Parametric tests and their assumptions	39
	3.2	Decision errors	42
		3.2.1 Type I and II errors	42
		3.2.2 Asymmetry and scalable decision criteria	44
	3.3	Other testing methods	45
		3.3.1 Robust parametric tests	45
		3.3.2 Randomization (permutation) tests	45
		3.3.3 Rank-based non-parametric tests	46
	3.4	Multiple testing	48
		3.4.1 The problem	48
		3.4.2 Adjusting significance levels and/or P values	49
	3.5	Combining results from statistical tests	50
		3.5.1 Combining P values	50
		3.5.2 Meta-analysis	50
	3.6	Critique of statistical hypothesis testing	51
		3.6.1 Dependence on sample size and stopping rules	51
		3.6.2 Sample space - relevance of data not observed	52
		3.6.3 P values as measure of evidence	53
		3.6.4 Null hypothesis always false	53
		3.6.5 Arbitrary significance levels	53
		3.6.6 Alternatives to statistical hypothesis testing	53
	3.7	Bayesian hypothesis testing	54
4	G	Graphical exploration of data	58
	4.1	Exploratory data analysis	58
		4.1.1 Exploring samples	58
	4.2	Analysis with graphs	62
		4.2.1 Assumptions of parametric linear models	62
	4.3	Transforming data	64
		4.3.1 Transformations and distributional assumptions	65
		4.3.2 Transformations and linearity	67
		4.3.3 Transformations and additivity	67
		Standardizations	67
		Outliers	68
	4.6	Censored and missing data	68
		4.6.1 Missing data	68
		4.6.2 Censored (truncated) data	69
	4.7	General issues and hints for analysis	71
		471 Conoral issues	71

5	10	Correlation and regression	72
	5.1	Correlation analysis	72
		5.1.1 Parametric correlation model	72
		5.1.2 Robust correlation	76
		5.1.3 Parametric and non-parametric confidence regions	76
	5.2	Linear models	77
	5.3	Linear regression analysis	78
		5.3.1 Simple (bivariate) linear regression	78
		5.3.2 Linear model for regression	80
		5.3.3 Estimating model parameters	85
		5.3.4 Analysis of variance	88
		5.3.5 Null hypotheses in regression	89
		5.3.6 Comparing regression models	90
		5.3.7 Variance explained	91
		5.3.8 Assumptions of regression analysis	92
		5.3.9 Regression diagnostics	94
		5.3.10 Diagnostic graphics	96
		5.3.11 Transformations	98
		5.3.12 Regression through the origin	98
		5.3.13 Weighted least squares	99
		5.3.14 X random (Model II regression)	100
		5.3.15 Robust regression	104
		Relationship between regression and correlation	106
	5.5	Smoothing	107
		5.5.1 Running means	107
		5.5.2 LO(W)ESS	107
		5.5.3 Splines	108
		5.5.4 Kernels	108
		5.5.5 Other issues	109
		Power of tests in correlation and regression	109
	5.7	General issues and hints for analysis	110
		5.7.1 General issues	110
		5.7.2 Hints for analysis	110
5	M	Iultiple and complex regression	111
	6.1	Multiple linear regression analysis	111
		6.1.1 Multiple linear regression model	114
		6.1.2 Estimating model parameters	119
		6.1.3 Analysis of variance	119
		6.1.4 Null hypotheses and model comparisons	121
		6.1.5 Variance explained	122
		6.1.6 Which predictors are important?	122
		6.1.7 Assumptions of multiple regression	124
		6.1.8 Regression diagnostics	125
		6.1.9 Diagnostic graphics	125
		6.1.10 Transformations	127
		6.1.11 Collinearity	127

6.1.12 Interactions in multiple regression	130
6.1.13 Polynomial regression	133
6.1.14 Indicator (dummy) variables	135
6.1.15 Finding the "best" regression model	137
6.1.16 Hierarchical partitioning	141
6.1.17 Other issues in multiple linear regression	142
6.2 Regression trees	143
6.3 Path analysis and structural equation modeling	145
6.4 Nonlinear models	150
6.5 Smoothing and response surfaces	152
6.6 General issues and hints for analysis	153
6.6.1 General issues	153
6.6.2 Hints for analysis	154
7 Design and power analysis	155
7.1 Sampling	155
7.1.1 Sampling designs	155
7.1.2 Size of sample	157
7.2 Experimental design	157
7.2.1 Replication	158
7.2.2 Controls	160
7.2.3 Randomization	161
7.2.4 Independence	163
7.2.5 Reducing unexplained variance	164
7.3 Power analysis	164
7.3.1 Using power to plan experiments (a priori power analysis)	166
7.3.2 Post hoc power calculation	168
7.3.3 The effect size	168
7.3.4 Using power analyses	170
7.4 General issues and hints for analysis	171
7.4.1 General issues	171
7.4.2 Hints for analysis	172
8 Comparing groups or treatments – analysis of variance	173
8.1 Single factor (one way) designs	173
8.1.1 Types of predictor variables (factors)	176
8.1.2 Linear model for single factor analyses	178
8.1.3 Analysis of variance	184
8.1.4 Null hypotheses	186
8.1.5 Comparing ANOVA models	187
8.1.6 Unequal sample sizes (unbalanced designs)	187
8.2 Factor effects	188
8.2.1 Random effects: variance components	188
8.2.2 Fixed effects	190
8.3 Assumptions	191
8.3.1 Normality	192
8.3.2 Variance homogeneity	193
8.3.3 Independence	193

8.4	ANOVA diagnostics	194
	Robust ANOVA	195
	8.5.1 Tests with heterogeneous variances	195
	8.5.2 Rank-based ("non-parametric") tests	195
	8.5.3 Randomization tests	196
8.6	Specific comparisons of means	196
	8.6.1 Planned comparisons or contrasts	197
	8.6.2 Unplanned pairwise comparisons	199
	8.6.3 Specific contrasts versus unplanned pairwise comparisons	201
8.7	Tests for trends	202
8.8	Testing equality of group variances	203
8.9	Power of single factor ANOVA	204
8.10	General issues and hints for analysis	206
	8.10.1 General issues	206
	8.10.2 Hints for analysis	206
9 6	lultifactor analysis of variance	208
9.1	Nested (hierarchical) designs	208
	9.1.1 Linear models for nested analyses	210
	9.1.2 Analysis of variance	214
	9.1.3 Null hypotheses	215
	9.1.4 Unequal sample sizes (unbalanced designs)	216
	9.1.5 Comparing ANOVA models	216
	9.1.6 Factor effects in nested models	216
	9.1.7 Assumptions for nested models	218
	9.1.8 Specific comparisons for nested designs	219
	9.1.9 More complex designs	219
	9.1.10 Design and power	219
9.2	Factorial designs	221
	9.2.1 Linear models for factorial designs	225
	9.2.2 Analysis of variance	230
	9.2.3 Null hypotheses	232
	9.2.4 What are main effects and interactions really measuring?	237
	9.2.5 Comparing ANOVA models	241
	9.2.6 Unbalanced designs	241
	9.2.7 Factor effects	247
	9.2.8 Assumptions	249
	9.2.9 Robust factorial ANOVAs	250
	9.2.10 Specific comparisons on main effects	250
	9.2.11 Interpreting interactions	25:
	9.2.12 More complex designs	255
	9.2.13 Power and design in factorial ANOVA	259
	Pooling in multifactor designs	260
	Relationship between factorial and nested designs	26
9.5	General issues and hints for analysis	26
	9.5.1 General issues	26
	9.5.2 Hints for analysis	26

10	Randomized blocks and simple repeated measures:	
	unreplicated two factor designs	262
10	Unreplicated two factor experimental designs	262
10.	10.1.1 Randomized complete block (RCB) designs	262
	10.1.2 Repeated measures (RM) designs	265
10	2 Analyzing RCB and RM designs	268
10.	10.2.1 Linear models for RCB and RM analyses	268
	10.2.2 Analysis of variance	272
	10.2.3 Null hypotheses	273
	10.2.4 Comparing ANOVA models	274
10	3 Interactions in RCB and RM models	274
10.	10.3.1 Importance of treatment by block interactions	274
	10.3.2 Checks for interaction in unreplicated designs	277
10	4 Assumptions	280
10.	10.4.1 Normality, independence of errors	280
	10.4.2 Variances and covariances - sphericity	280
	10.4.3 Recommended strategy	284
10	5 Robust RCB and RM analyses	284
	6 Specific comparisons	285
	7 Efficiency of blocking (to block or not to block?)	285
	8 Time as a blocking factor	287
	9 Analysis of unbalanced RCB designs	287
	O Power of RCB or simple RM designs	289
	1 More complex block designs	290
10.1	10.11.1 Factorial randomized block designs	290
	10.11.2 Incomplete block designs	292
	- · · · · · · · · · · · · · · · · · · ·	292
	10.11.3 Latin square designs 10.11.4 Crossover designs	296
10 1	2 Generalized randomized block designs	298
	3 RCB and RM designs and statistical software	298
	4 General issues and hints for analysis	299
10.1	10.14.1 General issues	299
	10.14.1 General issues 10.14.2 Hints for analysis	300
	10.14.2 fullts 101 alialysis	500
11	Split-plot and repeated measures designs: partly nested	
• •	analyses of variance	301
11	1 Portly poeted designs	301
11	.1 Partly nested designs 11.1.1 Split-plot designs	301
	11.1.1 Spht-plot designs 11.1.2 Repeated measures designs	305
	11.1.2 Repeated measures designs 11.1.3 Reasons for using these designs	309
11	.2 Analyzing partly nested designs	309
11	11.2.1 Linear models for partly nested analyses	310
	11.2.2 Analysis of variance	313
	11.2.3 Null hypotheses	315
	11.2.4 Comparing ANOVA models	318
11	3 Assumptions	318
11	11.3.1 Between plots/subjects	318
	11.3.2 Within plots/subjects and multisample sphericity	318
	TION MITTHE MODIFICATION MET METERS AND ACTIONS	510

11.4	Robust partly nested analyses	320
11.5	Specific comparisons	320
	11.5.1 Main effects	320
	11.5.2 Interactions	321
	11.5.3 Profile (i.e. trend) analysis	321
11.6	Analysis of unbalanced partly nested designs	322
	Power for partly nested designs	323
11.8	More complex designs	323
	11.8.1 Additional between-plots/subjects factors	324
	11.8.2 Additional within-plots/subjects factors	329
	11.8.3 Additional between-plots/subjects and within-plots/	
	subjects factors	332
	11.8.4 General comments about complex designs	335
	Partly nested designs and statistical software	335
11.10	General issues and hints for analysis	337
	11.10.1 General issues	337
	11.10.2 Hints for individual analyses	337
12	Analyses of covariance	339
12.1	Single factor analysis of covariance (ANCOVA)	339
	12.1.1 Linear models for analysis of covariance	342
	12.1.2 Analysis of (co)variance	347
	12.1.3 Null hypotheses	347
	12.1.4 Comparing ANCOVA models	348
12.2	Assumptions of ANCOVA	348
	12.2.1 Linearity	348
	12.2.2 Covariate values similar across groups	349
	12.2.3 Fixed covariate (X)	349
12.3	Homogeneous slopes	349
	12.3.1 Testing for homogeneous within-group regression slopes	349
	12.3.2 Dealing with heterogeneous within-group regression	
	slopes	350
	12.3.3 Comparing regression lines	352
	Robust ANCOVA	352
	Unequal sample sizes (unbalanced designs)	353
12.6	Specific comparisons of adjusted means	353
	12.6.1 Planned contrasts	353
	12.6.2 Unplanned comparisons	353
12.7	More complex designs	353
	12.7.1 Designs with two or more covariates	353
	12.7.2 Factorial designs	354
	12.7.3 Nested designs with one covariate	355
	12.7.4 Partly nested models with one covariate	356
12.8	General issues and hints for analysis	357
	12.8.1 General issues	357
	12.8.2 Hints for analysis	358

13	Generalized linear models and logistic regression	359
13.	1 Generalized linear models	359
13.	2 Logistic regression	360
	13.2.1 Simple logistic regression	360
	13.2.2 Multiple logistic regression	365
	13.2.3 Categorical predictors	368
	13.2.4 Assumptions of logistic regression	368
	13.2.5 Goodness-of-fit and residuals	368
	13.2.6 Model diagnostics	370
	13.2.7 Model selection	370
	13.2.8 Software for logistic regression	371
	3 Poisson regression	371
	4 Generalized additive models	372
13.	5 Models for correlated data	375
	13.5.1 Multi-level (random effects) models	376
	13.5.2 Generalized estimating equations	377
13.	6 General issues and hints for analysis	378
	13.6.1 General issues	378
	13.6.2 Hints for analysis	379
14	Analyzing frequencies	380
14.	1 Single variable goodness-of-fit tests	381
14.	2 Contingency tables	381
	14.2.1 Two way tables	381
	14.2.2 Three way tables	388
14.	3 Log-linear models	393
	14.3.1 Two way tables	394
	14.3.2 Log-linear models for three way tables	395
	14.3.3 More complex tables	400
14.	4 General issues and hints for analysis	400
	14.4.1 General issues	400
	14.4.2 Hints for analysis	400
15	Introduction to multivariate analyses	401
15.	1 Multivariate data	401
15.	2 Distributions and associations	402
15.	3 Linear combinations, eigenvectors and eigenvalues	405
	15.3.1 Linear combinations of variables	405
	15.3.2 Eigenvalues	405
	15.3.3 Eigenvectors	406
	15.3.4 Derivation of components	409
15.	4 Multivariate distance and dissimilarity measures	409
	15.4.1 Dissimilarity measures for continuous variables	412
	15.4.2 Dissimilarity measures for dichotomous (binary) variables	413
	15.4.3 General dissimilarity measures for mixed variables	413
	15.4.4 Comparison of dissimilarity measures	414
15	5 Comparing distance and/or dissimilarity matrices	414

15.6	5 Data standardization	415
15.7	Standardization, association and dissimilarity	417
15.8	Multivariate graphics	417
15.9	Screening multivariate data sets	418
	15.9.1 Multivariate outliers	419
	15.9.2 Missing observations	419
15.10	General issues and hints for analysis	423
	15.10.1 General issues	423
	15.10.2 Hints for analysis	424
16	Multivariate analysis of variance and discriminant analysis	425
16,1	Multivariate analysis of variance (MANOVA)	425
	16.1.1 Single factor MANOVA	426
	16.1.2 Specific comparisons	432
	16.1.3 Relative importance of each response variable	432
	16.1.4 Assumptions of MANOVA	433
	16.1.5 Robust MANOVA	434
	16.1.6 More complex designs	434
16.2	2 Discriminant function analysis	435
	16.2.1 Description and hypothesis testing	437
	16.2.2 Classification and prediction	439
	16.2.3 Assumptions of discriminant function analysis	441
	16.2.4 More complex designs	441
16.3	B MANOVA vs discriminant function analysis	441
16.4	General issues and hints for analysis	441
	16.4.1 General issues	441
	16.4.2 Hints for analysis	441
17	Principal components and correspondence analysis	443
17.1	Principal components analysis	443
	17.1.1 Deriving components	447
	17.1.2 Which association matrix to use?	450
	17.1.3 Interpreting the components	451
	17.1.4 Rotation of components	451
	17.1.5 How many components to retain?	452
	17.1.6 Assumptions	453
	17.1.7 Robust PCA	454
	17.1.8 Graphical representations	454
	17.1.9 Other uses of components	456
17.2	2 Factor analysis	458
	3 Correspondence analysis	459
	17.3.1 Mechanics	459
	17.3.2 Scaling and joint plots	461
	17.3.3 Reciprocal averaging	462
	17.3.4 Use of CA with ecological data	462
	17.3.5 Detrending	463
17.4	Canonical correlation analysis	463

xiv

17.5 Redundancy analysis	466
17.6 Canonical correspondence analysis	467
17.7 Constrained and partial "ordination"	468
17.8 General issues and hints for analysis	471
17.8.1 General issues	471
17.8.2 Hints for analysis	471
18 Multidimensional scaling and cluster analysis	473
18.1 Multidimensional scaling	473
18.1.1 Classical scaling - principal coordinates analysis (PCoA)	474
18.1.2 Enhanced multidimensional scaling	476
18.1.3 Dissimilarities and testing hypotheses about groups of	
objects	482
18.1.4 Relating MDS to original variables	487
18.1.5 Relating MDS to covariates	487
18.2 Classification	488
18.2.1 Cluster analysis	488
18.3 Scaling (ordination) and clustering for biological data	491
18.4 General issues and hints for analysis	493
18.4.1 General issues	493
18.4.2 Hints for analysis	493
19 Presentation of results	494
19.1 Presentation of analyses	494
19.1.1 Linear models	494
19.1.2 Other analyses	497
19.2 Layout of tables	497
19.3 Displaying summaries of the data	498
19.3.1 Bar graph	500
19.3.2 Line graph (category plot)	502
19.3.3 Scatterplots	502
19.3.4 Pie charts	503
19.4 Error bars	504
19.4.1 Alternative approaches	506
19.5 Oral presentations	507
19.5.1 Slides, computers, or overheads?	507
19.5.2 Graphics packages	508
19.5.3 Working with color	508
19.5.4 Scanned images	509
19.5.5 Information content 19.6 General issues and hints	509
15.0 General issues and nints	510
References	511
Index	527