

Analysis of Quantitative data Linear and non-linear relationships

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Association between 2 continuous variables Linear relationship

Signal-to-noise ratio



Signal
Noise= statistical significanceSignal
Noise= no statistical significanceNoise

Signal-to-noise ratio and Correlation



• Signal is **similarity** of behaviour between variable x and variable y.



Correlation

- Most widely-used correlation coefficient:
 - Pearson product-moment correlation coefficient "r"
 - The magnitude and the direction of the relation between 2 variables
 - It is designed to range in value between -1 and +1
 - -0.6 < r > +0.6 : exciting

C <u>oefficient</u> (+ <u>ve</u> or <u>-ve</u>)	Strength of the relationship
0.0 to 0.2	Negligible
0.2 to 0.4	Weak
0.4 to 0.7	Moderate
0.7 to 0.9	Strong
0.9 to 1.0	Very strong

- Coefficient of determination "r²"
 - It gives the proportion of variance in Y that can be explained by X (in percentage).
 - It helps with the interpretation of r
 - It's basically the effect size

Correlation



Coefficient of determination



How big is the difference?

95% confidence interval

R squared (eta squared)

Mean of column A

Mean of column B

89.71

92.06

0 4964 to 5.185 0.03107

Difference between means (B - A) ± SEM 2.344 ± 1.428

13 14

15

16 17

18

19

00

5 cell lines on protein expression

	AL MA) =		- 1		1	ANOVA results		0
			8	Ordinary one-way ANOVA			- 4			-
	Unpaired t test			ANOVA results			1	Table Analyzed	Neutrophils	
_	4			1			2			
1	Table Analyzed	coyotes	1	Table Analyzed	Transform	n of Protein expression	3	Repeated measures ANOVA summary		
2			2	Data sots analyzed	A_E		4	Assume sphericity?	No	
3	Column B	Males	2	Data sets analyzed	~-L		5	F	28.57	
4	VS.	VS.	3				6	P value	0.0002	
5	Column A	Females	4	ANOVA summary			7	P value summary	***	
6			5	F	8.127		0	Statistically significant ($B < 0.05$)2	Voc	
7	Unpaired t test		6	P value	<0.0001		0		165	
8	P value	0.1045	-	Dualua aumanani	****		<u></u>	(seisser-(sreennouse's epsilon	0.6916	000/
9	P value summary	ns	1	P value summary			10	R square	0.8772	88%
10	Significantly different (P < 0.05)?	No	8	Significant diff_among means (P < 0.05)? Yes	210/	11		+	
11	One- or two-tailed P value?	Two-tailed	9	R square	0.3081	5 1%				
12	t, df	t=1.641, df=84								

	1	2way ANOVA ANOVA results	Be	er go	oggles eff	ect		
		1						
85	1	Table Analyzed	data for 2-way					
20/	2							
3%	3	Two-way ANOVA	Ordinary					
	4	Alpha	0.05					
	5							
	6	Source of Variation	% of total variation	P value	P value summary	Significant?		
	7	Interaction	22.06	<0.0001	****	Yes		
	8	Alcohol Consumption	37.16	<0.0001	****	Yes 22	%+37%	%+1.88% = 61%
	9	Gender	1.882	0.1614	ns	No		
	10							
	11	ANOVA table	SS	DF	MS	F (DFn, DFd)	P value	
	12	Interaction	1978	2	989.1	F (2, 42) = 11.91	P<0.0001	
	13	Alcohol Consumption	3332	2	1666	F (2, 42) = 20.07	P<0.0001	
	14	Gender	168.8	1	168.8	F (1, 42) = 2.032	P=0.1614	
	15	Residual	3488	42	83.04			
	16							

Correlation: Two more things

Thing 1: Pearson correlation is a parametric test First assumption for parametric test: Normality **Correlation: bivariate Gaussian distribution**



Symmetry-ish of the values on either side of the line of best fit.

Correlation: Two more things

Thing 2: Line of best fit comes from a regression Correlation: nature and strength of the association Regression: nature and strength of the association <u>and prediction</u>



Correlation treelight.xlsx



Amount of light in a tree

Have a go!

Correlation with Prism 8

Х

Pulk in analysis		Compute r for X vs. every Y data set:
Which analysis	Analyze which dat	O Compute r between two selected data sets:
 Transform, Normalize Transform Transform concentrations (X) Normalize Prune rows Remove baseline and column math Transpose X and Y Fraction of total XY analyses Nonlinear regression (curve fit) Linear regression Fit spline/LOWESS Smooth, differentiate or integrate curve Area under curve Deming (Model II) linear regression Row means with SD or SEM Correlation Interpolate a standard curve Column analyses Grouped analyses 	When you analy: than one data - which da	X Column A : Variable 2 Assume data are sampled from Gaussian distribution? Image: State of the system of the correlation coefficients. No. Compute Pearson correlation coefficients. No. Compute nonparametric Spearman correlation. Options P value: One-tailed Image: One-tailed Image: One-tailed Confidence interval: 95% Output Show this many significant digits (for everything except P values): P value style: GP: 0.1234 (ns), 0.0332 (*), 0.0021 (**), Image: One-tailed Graphing Image: Oreate a heatmap of the correlation matrix.
Contingency table analyses Contingency table analyses		Learn Cancel

Parameters: Correlation



Correlation with Prism 8



Correlation with Prism 8 (if you also want a line of best fit)



Pa	rameters: Linear Regression						
	Interpolate						
	Interpolate unknowns from standard curve						
	Compare						
	Test whether slopes and intercepts are significantly different						
	Graphing options						
	\square Show the 95% confidence bands \lor of the best-fit line						
	Residual plot						
	Constrain						
	Force the line to go through $X = 0$, $Y = 0$						
	Replicates						
	Consider each replicate Y value as an individual point						
	Only consider the mean Y value of each point						
	Also calculate						
Test departure from linearity with runs test							
95% confidence interval of Y when X = 0							
	95% confidence interval of X when Y = 0						
	Range						
	Start regression line at: End regression line at:						
	OX= 0.6953660€ O						
	Show this many significant digits (for everything except P values):						
	P value style: GP: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0 🗸 🛛 = 6 🔺						
	Make these choices as default for future regressions						
	More choices Learn Cancel OK						

#	Tabular results	Light	
,		Ligit	Reculto
4	Post fit volues		ncsurs
	Olace	000.0	
2	Slope	-292.2	
3	Y-intercept	5014	
4	X-intercept	17.16	
)	1/slope	-0.003423	
5			
7	Std. Error		
B	Slope	55.41	
9	Y-intercept	342.2	
0			
1	95% Confidence Intervals		
2	Slope	-414.1 to -170.2	
3	Y-intercept	4261 to 5767	
4	X-intercept	13.59 to 25.66	
5			
6	Goodness of Fit		
7	R square	0.7165	
8	Sy.x	560.7	
9			
0	Is slope significantly non-zero?		
1	F	27.80	
2	DEn. DEd	1. 11	
3	P value	0.0003	
4	Deviation from zero?	Significant	Prediction
5			
6	Equation	$Y = -292 2 \times X + 5014$	

Correlation with Prism 8

Amount of light in a tree



Association between 2 continuous variables Non-linear relationship

Curve fitting

• Dose-response curves

- Nonlinear regression
- Dose-response experiments typically use around 5-10 doses of agonist, equally spaced on a logarithmic scale
- Y values are responses
- The aim is often to determine the IC50 or the EC50
 - IC50 (I=Inhibition): concentration of an agonist that provokes a response half way between the maximal (Top) response and the maximally inhibited (Bottom) response.
 - EC50 (E=Effective): concentration that gives half-maximal response



ſ	Pa	irametei	rs: Nonlin	ear Regres:	sion						×	
		Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag	1

Step by step analysis and considerations:

1- Choose a Model:

- 2- Choose a Method
- 3- Compare different conditions



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		Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag	

Step by step analysis and considerations:

1- Choose a Model

del	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag
009	se an equ	uation							
Ŧ	Recent	y used					^	New 1	
+	Standa	rd curves	to interpol	ate					
	Dose-re	sponse -	Stimulatio	n					
	log(a	gonist) vs.	response (th	nree parameter	s)				
	log(a	gonist) vs.	response	Variable slope (four para	meters)			
	log(a	gonist) vs.	normalized r	esponse					
	log(a	gonist) vs.	normalized r	esponse Vari	able slope	e			
	[Ago	nist] vs. res	ponse (thre	e parameters)					
	[Ago	nist] vs. res	ponse Var	riable slope (for	ur parame	eters)			
	[Ago	nist] vs. noi	malized resp	oonse					
	[Ago	nist] vs. nor	malized resp	oonse Variabi	le slope				
+	Dose-re	sponse -	Inhibition						
+	Dose-re	esponse -	Special, X i	s concentrat	ion				
÷	Dose-re	sponse -	Special, X i	s log(concen	tration)				
+	Binding	- Saturati	ion						
÷	Binding	- Compet	itive						
÷	Binding	- Kinetics							
÷	Enzyme	kinetics -	Inhibition						
+	Enzyme	kinetics -	Velocity a	s a function	of subst	rate	~		
-	-								
	2	10							
Re	cently use	:d							
ter	polate								
	oterpolate	unknowns	from standa	rd curve. Conf	dence int	erval	None V		
	riterpolate	UNADWIS	IT UIT Stariua	ru curve, com	uence int	ervai.	NUME		

ſ	Pa	arameter	rs: Nonlin	ear Regres	sion						2	
	$\left[\right]$	Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag	

Step by step analysis and considerations:

2- Choose a Method:

Paramet	ers: Nonli	near Regre	ssion			Group	R		(are	×		
Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag			
Outlie	ers											
ON	o special h	andling of o	utliers									
OD	etect and	eliminate ou	tliers	- 1 %	КГ	Create :	a table of dea	n data (with				
R	eport the p	presence of	outliers	- 1 7	UL	outliers	removed)	in uata (with				
Fittin	Fitting method											
۰Le	east squar	es regressio	n. Used mos	t commonly.								
	obust regr	ession. Out	iers have lit	le impact.								
OP	O Poisson regression. Y values are counts of objects or events.											
OD	on't fit the	curve. Inst	ead plot the	curve defined	by the in	itial value:	s of the paran	neters.				
Conv	ergence	criteria										
How	strict	Medium	~	🗹 Automati	cally swit	ch to stric	t convergence	e when neede	d			
Maxir	mum numb	er of iteratio	ons	1000								
Weig	hting me	thod										
٥N	o weightin	g. Minimize	the sum-of-	squares of the	distance	s of the p	oints from the	curve.				
Cł	hoose whe le value of	n you expe	ct the avera	ge distance bei	tween po	ints and c	urve to be un	related to				
OW	eight by 1	/Y^2. Minim	ize the sum	of the squares	of the re	lative dist	ance of the p	oints from the	e curve.			
C	hoose whe	n you expe	ct the avera	ge distance bei	tween po	ints and c	urve to be pro	oportional to 1	۲.			
Ow	/eight by	1/Y	~ K=	= 2								
Repli	cates											
00	onsider ea	ch replicate	Y value as a	n individual poi	nt							
00	Only consider the mean Y value of each point											
						Learn	Car	rel	OK			
						CCGITT	Ca		UN			

ſ	Pa	irametei	s: Nonlin	ear Regres:	sion						×	
		Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag	

Step by step analysis and considerations:

3- **Compare** different conditions:

lodel	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag
	a question lo compari- for each da Do the besis for each da Does one c Doarison m Akaike's Inf nost likely f Extra sum- Gelect the s	are you a son ata set, whi t-fit values of ata set, doe urve adequ acthod formation Ch to have gen of-squares is simpler mode	ch of two ed of selected u is the best-f ately fit all t riterion (AIC ierated the F test el unless the	quations (model unshared paran it value of a pa he data sets? c). Select the n data. P value is less	s) fits be neters dif rameter o nodel tha than [st? fer betwe differ fron t is t is v 0.05	en data sets? n a hypothetic f one fit is aml lagged, choos vithout formal	al value? biguous or e the other comparison	
	Bottom Top LogEC50 HillSlope	more par	ameters	Compare indeper elected parame f you select on arameter diffe parameters, you all the data sets	endent fit eter(s). e parame rs among u are ask	s with a g eter, you a data sets ing wheth	lobal fit that s are asking whe s. If you select er one curve a	hares the ther that t all the adequately fit	5
		Desel	act All						

ſ	Par	ramete	rs: Nonlin	ear Regres	sion						<u> </u>	3
	ſ	Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag	1

Step by step analysis and considerations:

1- Choose a Model:

not necessary to normalise

should choose it when values defining 0 and 100 are precise

variable slope better if plenty of data points (variable slope or 4 parameters)

2- Choose a Method: outliers, fitting method, weighting method and replicates

3- Compare different conditions:



4- Constrain:

depends on your experiment depends if your data don't define the top or the bottom of the curve



ſ	Parameters: Nonlinear Regression									×		
		Model	Method	Compare	Constrain	Initial values	Range	Output	Confidence	Diagnostics	Flag	Ъ

Step by step analysis and considerations:

5- Initial values:

defaults usually OK unless the fit looks funny

6- Range:

defaults usually OK unless you are not interested in the x-variable full range (ie time)

7- Output:

summary table presents same results in a ... summarized way.

8 – Confidence: calculate and plot confidence intervals

9- Diagnostics:

check for normality (weights) and outliers (but keep them in the analysis) check Replicates test residual plots



Curve fitting





LogEC50 -	-7.144 to -6.917	-6.064 to -5.848
EC50 7	7.179e-008 to 1.209e-007	8.633e-007 to 1.420e-006

R square	0.9476	0.9568

Curve fitting



Association between 2 continuous variables Linear relationship Diagnostics: Goodness-of-fit

- **Question**: Is there a relationship between time spent revising (Revise) and exam anxiety (Anxiety)? And, if yes, are boys and girls different?
- **Focus**: how good is the model?



Set of 5 clues

Clue 1: Graphical exploration of the data: linearity

Clue 2: Identification of outliers

Clue 3: R², the Coefficient of determination

Clue 4: Distribution of residuals with statistical tests

Clue 5: Distribution of residuals with a QQ plot

• Clue 1: Graphical exploration of the data



Clue 2: Identification of outliers



Categorical x and continuous y

• Clue 3: Coefficient of determination



• Distribution of the residuals



- **Clue 4**: Distribution of the residuals **with statistical tests**
- <u>Statistical tests</u>: **significant departure from normality**?
 - Anderson-Darling:
 - cumulative distribution different from a normal one?

- D'Agostino-Pearson:
 - asymmetry and shape different from normal distribution?
- Other tests: Shapiro-Wilk and Kolmogorov-Smirnov





• Clue 5: Distribution of the residuals with QQ plot



Normal QQ plot = Quantile – Quantile plot

Goodness of fit: let's do it exam anxiety.xlsx (Don't need 'Exam')



Association between time spent revising and exam anxiety. Are we getting it right?

Goodness of fit: let's do it exam anxiety.xlsx (Don't need 'Exam')

Excel File

1	Α	В	С	D	E
1	Code	Revise	Exam	Anxiety	Gender
2	2	11	65	88.716	Female
3	6	22	70	60.506	Female
4	7	16	20	81.462	Female
5	8	21	55	75.82	Female
5	9	25	50	69.372	Female
7	10	18	40	82.268	Female
3	14	18	50	75.014	Female
Э	18	29	95	79.044	Female
0	19	4	50	91.134	Female
1	23	22	85	65.342	Female
2	24	84	90	0.056	Female
3	25	23	30	71.79	Female
4	26	26	60	81.462	Female
5	28	72	75	27.46	Female
6	29	37	27	73.402	Female
7	31	3	75	89.522	Female
8	32	36	90	75.014	Female
9	36	9	10	79.044	Female
0	39	12	5	83.074	Female
1	42	8	45	78.238	Female
2	44	22	70	74.208	Female
3	45	21	50	75.82	Female
4	50	19	50	73.402	Female
5	51	0	35	93.552	Female
6	52	52	80	58.894	Female
7	53	38	50	53.252	Female
8	55	23	75	89.522	Female
9	56	11	25	71.79	Female
0	60	42	70	68.566	Female

Prism File

Table format:		X	Group A	Group B
	~	Revise	Anxiety F	Anxiety M
-	×	X	Y	Y
31	64	11	81.462	
32	67	4	91.940	
33	68	28	86.298	
34	70	29	63.730	
35	72	16	71.790	
36	74	10	84.686	
37	76	8	77.432	
38	77	5	82.268	
39	79	38	50.834	
40	82	6	84.686	
41	83	68	20.206	
42	85	1	83.880	
43	87	42	95.970	
44	88	13	62.118	
45	91	5	84.686	
46	92	12	83.074	
47	94	2	87.910	
8	97	15	84.686	
49	99	13	70.984	
50	100	14	78.238	
51	103	20	91.134	
52	1	4		86.298
53	3	27		70.178
54	4	53		61.312
55	5	4		89.522
56	11	18		79.044
57	12	16		80.656
58	13	13		70.178
59	15	98		34.714
60	16	1		95.164
61	17	14		75.820
62	20	23		64.536
63	21	14		80.656
64	22	12		77.432

Goodness of fit with Prism 8

Decemptors: Nonlinear Pagrossian		ц,	
Parameters: Nonimear Regression	×		
Model Method Compare Constrain	Group DI Group El Group El Group Gl Group HL G Parameters: Nonlinear Regression		Parameters: Nonlinear Regression ×
Choose an equation Binding - Saturation Competitive Kinetics inetics - Inhibition Exponential Clines Straight line Line through point (X0, Y0) Line through origin Horizontal line Semilog line X is log, Y is ling Semilog line X is log, Y is ling Semilog line X is ling, Y is ling Semilog line X and Y both I Segmental linear regression Continuous hinge function, Peril Cumulative Gaussian Peril	Parameters: Nonlinear Regression Model Method Compare Constrain Initial values Range I Outliers Clue 2 No special ha I	Parameters: Nonlinear Regression Model Method Compare Constrain Initial values Range C What question are you asking? No comparison For each data set, which of two equations (models) fits best? Image: Comparison For each data set, which of two equations (models) fits best? Image: Comparison Image: Comparison for each data set, does the best-fit value of a parameters differ Image: Comparison for each data set, does the best-fit value of a parameter differ Image: Comparison method Image: Comparison method Image: Comparison for each data set is most likely to have generated the data. Image: Comparison for each data set is select the simpler model unless the P value is less than Image: Compare independent fits with the select for each data set is select the simpler model unless the P value is less than	Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag How to quantify ge Clue 3 Sy.x Sum-of-Squares Adjusted R squared RMSE AICc Are residuals Gaussian (normal)? Anderson-Darling test Clue 4 O'Agostino-Pearson omnibus normality test Clue 4 Shapiro-Wilk normality test Clue 4 Shapiro-Wilk normality test Clue 4 Model statered or heteroscedastic? Runs test Replicates test No residual graph O No residual graph
Two intersecting lines. Fit the Same as linear regression, but with regression and automatically remover Straight line Analytical derivatives Interpolate Interpolate unknowns from standate	 Weighting method No weighting. Minimize the sum-of-squares of the distances Choose when you expect the average distance between poin the value of Y. Weight by 1/Y^2. Minimize the sum of the squares of the rela Choose when you expect the average distance between poin Weight by 1/Y & K = 2 Replicates Consider each replicate Y value as an individual point Only consider the mean Y value of each point 	Select All Deselect All	Residual vs Y plot Residual vs Y plot Homoscedasticity plot QQ plot Are the parameters inte Clue 5 Covariance of paramet Clue 5 Dependency Hougaard's measure of skewness Make these diagnostics choices the default for future fits.
Have a go!			

• **Clue 1**: Graphical exploration of the data: linear relationship



Goodness of fit with Prism 8

• **Clue 2**: Identification of outliers





• Clue 3: Coefficient of determination





• **Clue 4**: Distribution of the residuals **with statistical tests**



• Clue 5: Distribution of the residuals with QQ plot



Goodness of fit with Prism 8





There is a strong negative relationship between time spent revising and exam anxiety and that relationship is significantly stronger for girls than for boys (p=0.0056).