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January 20, 2021

Biol 520C: Statistical modelling for biological data

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Housekeeping

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 Datasets for papers: consider asking your supervisor or lab mates for data on your study system.





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Our verbal hypothesis in this case is 'Y is proportional to $X_1, X_2, ... X_m$ '. With multiple factors, how do we approach the problem statistically?

Simple Linear Regression



Simple Linear Regression



Remembering that for simple linear regression our data is of the form:

$$d = \{(x_1, y_1), (x_2, y_2), \dots, (x_n, y_n)\}$$

$$\begin{array}{ccc} X & Y \\ \hline x_1 & y_1 \\ x_2 & y_2 \\ \dots & \dots \\ x_n & y_n \end{array}$$

And our relationship is described by an intercept (β_0) and a slope (β_1) :

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$$

Multiple Regression Model



Multiple Regression Model



For m explanatory variables, the data are of the form:

Υ	X_1	X_2	 X_m
<i>y</i> ₁	X_{11}	X_{21}	 X_m1
y_1	X_{12}	X_{22}	 X_{m2}
y_n	X_{1n}	X_{2n}	 X_{mn}

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A linear regression with multiple explanatory variables is described by an intercept (β_0) and a regression coefficients $(\beta_1, \beta_2, \dots, \beta_m)$:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_m x_{mi} + \varepsilon_i$$





$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix}$$



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$$\beta = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{pmatrix}$$

$$\mathbf{x} = \begin{pmatrix} 1 & x_1 1 & x_2 1 \\ 1 & x_1 2 & x_2 2 \\ \vdots & \vdots & \vdots \\ 1 & x_1 n & x_2 n \end{pmatrix}$$



Given our dataset $(y_1, x_11, x_21), (y_2, x_12, x_22), \dots, (y_n, x_1n, x_2n)$ we can re-write the problem in matrix notation:

$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix} \qquad \beta = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{pmatrix} \qquad x = \begin{pmatrix} 1 & x_1 1 & x_2 1 \\ 1 & x_1 2 & x_2 2 \\ \vdots & \vdots & \vdots \\ 1 & x_1 n & x_2 n \end{pmatrix}$$

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Notice how the dimensions of the matrices all line up, so even though we've added new parameters we can still estimate their values using our old friend $\beta = (x^Tx)^{-1}x^Ty$.

Pre-analysis

Mult. Parameters, Mult. Interactions



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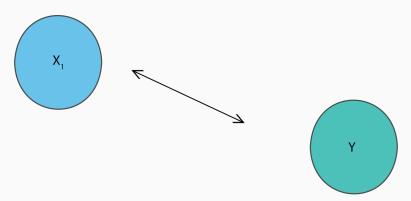


With only one parameter, there is only a single relationship that is possible

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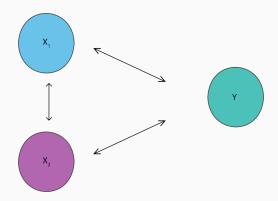


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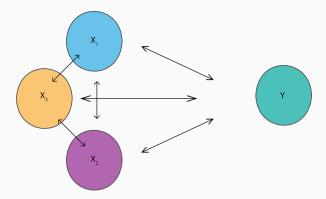




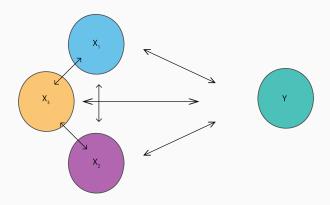
With two parameters, there are 3 relationship that are possible



With three parameters, there are 6 relationship that are possible



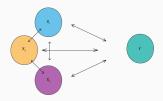
With three parameters, there are 6 relationship that are possible



... and it only gets worse as you add more parameters

Collinearity

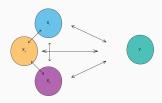




Collinearity



The challenge is that only some of the relationships are of interest, whereas others can introduce identifiability issues

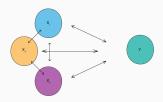


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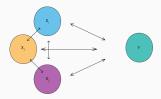
Collinearity



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Relationships between the predictors introduce the problem of collinearity







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In other words, parameter values for collinear predictors can't be trusted, which means we can't trust any of the predictions that the model makes.





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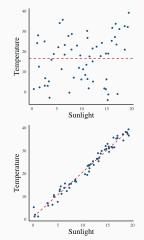
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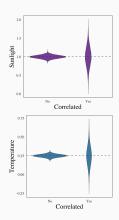
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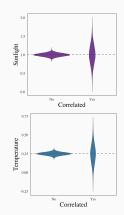
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We can test for the influence of correlated data on parameter estimates:



Note how some parameter estimates can even change signs!

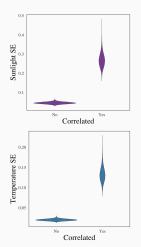
Collinearity and Standard Errors



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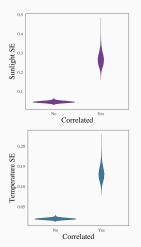
Collinearity also inflates standard errors



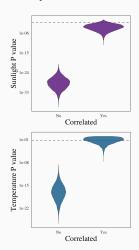
Collinearity and Standard Errors



Collinearity also inflates standard errors



... which means that p values are untrustworthy







• Do nothing.



- Do nothing.
- Collect more data.



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- Drop one of the correlated variables.



- Do nothing.
- Collect more data.
- Drop one of the correlated variables.
- Perform a Principal Component Analysis (PCA) on the data to reduce dimensionality.

Correction: Do nothing



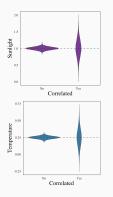
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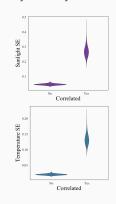


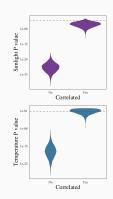
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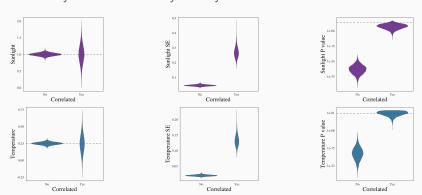




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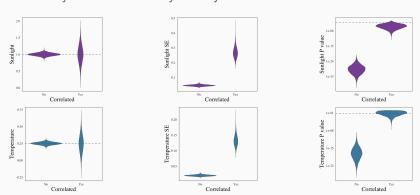
Collinearity doesn't necessarily mean your model is incorrect



... but it does mean you need to be careful when making inferences.



Collinearity doesn't necessarily mean your model is incorrect



... but it does mean you need to be careful when making inferences. Methods like cross-validation or sensitivity analyses should be used to confirm model fit and performance.





Collinearity doesn't bias the parameter estimates (i.e., parameter estimates are correct on average)

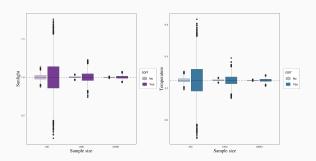


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With enough data, the problem of collinearity eventually goes away (terms asymptotic convergence).







Dropping collinear parameters can correct for the issue of collinearity



Dropping collinear parameters can correct for the issue of collinearity, at a cost of reducing your R-squared



Dropping collinear parameters can correct for the issue of collinearity, at a cost of reducing your R-squared and potentially reducing biological relevance.



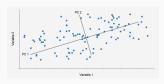


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We're not covering the details of PCAs, but, briefly, they reduce the dimensionality of a dataset by creating new uncorrelated variables that successively maximize variance.

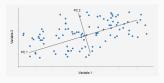


Source: statistiXL



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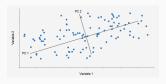
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Instead of fitting a model to your original data, you use the uncorrelated principal components in your analysis.



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Instead of fitting a model to your original data, you use the uncorrelated principal components in your analysis.

Analyses are robust to collinearity in the original data, but results can be more difficult to interpret.

Multiple linear regression in

action

Example: Ant richness



The Question: Do latitude and elevation have a measurable effect on ant species richness in forest plots in New England?

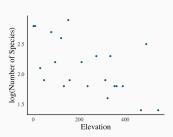
Gotelli, N.J. & Ellison, A.M. (2002). Biogeography at a regional scale: determinants of ant species density in bogs and forests of New England. Ecology, 83, 1604–1609



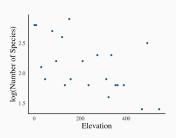


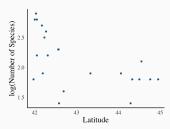
Number of Species	Latitude	Elevation
1.8	41.97	389
2.8	42	8
2.9	42.03	152
2.8	42.05	1
2.2	42.05	210
2.7	42.17	78
1.9	42.19	47
2.5	42.23	491
2.6	42.27	121
2.2	42.31	95
2.3	42.56	274
2.3	42.57	335
1.4	42.58	543
1.6	42.69	323
1.9	43.33	158
1.9	44.06	313
1.4	44.29	468
1.8	44.33	362
1.8	44.5	236
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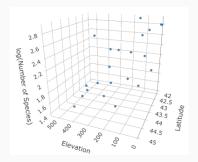
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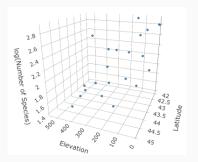
The relationships between richness, latitude, and elevation play out in multiple dimensions.



Ant richness: The data cont.



The relationships between richness, latitude, and elevation play out in multiple dimensions.



The first step is to check for any relationship between elevation and latitude

Ant Richness: Pre-analysis



Ant Richness: Pre-analysis



We can also do this by using the lm() function:

```
data <- read.csv("Ant_Richness.csv")
preFIT <- lm(latitude ~ elevation, data = data)
summary(preFIT)</pre>
```

Ant Richness: Pre-analysis

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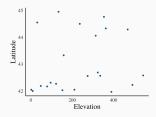
summary(preFIT)

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We can also do this by using the lm() function:

```
Call:
lm(formula = latitude ~ elevation, data = data)
Residuals:
    Min
             10 Median
                                    Max
-1.2357 -0.7383 -0.5589 0.9789 2.0485
Coefficients:
             Estimate Std. Error t value Pr(>t)
(Intercept) 42.743402
                      0.411929 103.764
                                           <2e-16 ***
elevation
             0.001189
                        0.001461
                                   0.814
                                          0.425
Residual standard error: 1.091 on 20 degrees of freedom
Multiple R-squared: 0.03205, Adjusted R-squared: -0.01635
F-statistic: 0.6622 on 1 and 20 DF, p-value: 0.4254
```



Ant richness: matrix notation



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There was no significant relationship between our two predictors (Elevation and Latitude) so we can continue with our analyses.



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The regression problem in matrix notation is:

$$\begin{pmatrix} 1.8 \\ 2.8 \\ \vdots \\ 1.8 \end{pmatrix} = \begin{pmatrix} 1 & 41.97 & 389 \\ 1 & 42.00 & 8 \\ \vdots & \vdots & & \\ 1 & 44.95 & 133 \end{pmatrix} \cdot \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \end{pmatrix} + \begin{pmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{pmatrix}$$

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Because the dimensions of the matrices are all compatible we can estimate the parameters values using $\beta = (x^Tx)^{-1}x^Ty$.

Ant Richness: Est. the parameters



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y <- matrix(data$num_sp,
            nrow = nrow(data), ncol = 1)
xtx <- t(x) %*% x
xtx.inv <- solve(xtx)
xty <- t(x) %*% y
beta <- xtx.inv %*% xty
heta
             [,1]
[1,] 11.115818937
Γ2.1 -0.201828913
Γ3.1 -0.001372863
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Again, this can easily done in R:

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xtx.inv <- solve(xtx)
xty <- t(x) %*% y
beta <- xtx.inv %*% xty
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FIT <- lm(num_sp ~ latitude + elevation, data = data)
```

Ant Richness: Analysis



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```
FIT <- lm(num_sp ~ latitude + elevation, data = data)
Call:
lm(formula = num_sp ~ latitude + elevation, data = data)
Residuals:
     Min
             10 Median 30
                                       Max
-0.63613 -0.21966 0.06166 0.17932 0.58149
Coefficients:
             Estimate Std. Error t value Pr(>t)
(Intercept) 11.1158189 2.7445783 4.050 0.000683 ***
latitude -0.2018289 0.0641510 -3.146 0.005318 **
elevation -0.0013729 0.0004259 -3.223 0.004473 **
Residual standard error: 0.3131 on 19 degrees of freedom
Multiple R-squared: 0.5653, Adjusted R-squared: 0.5196
F-statistic: 12.36 on 2 and 19 DF, p-value: 0.0003651
```



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FIT <- lm(num_sp ~ latitude + elevation, data = data)
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Call:

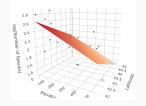
lm(formula = num_sp ~ latitude + elevation, data = data)

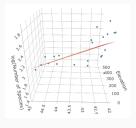
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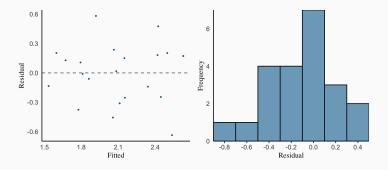
Ant richness: Residuals



After fitting the model it's important to check the residuals to check for the assumption of normality.



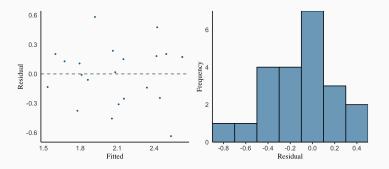
After fitting the model it's important to check the residuals to check for the assumption of normality.



Ant richness: Residuals



After fitting the model it's important to check the residuals to check for the assumption of normality.



We'll cover residuals in more detail next lecture, but for now suffice it to say these look pretty good.





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It's important to be very careful when adding parameters to a model.



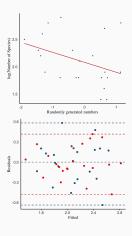


```
set.seed(84)
NOISE <- rnorm(22)
FIT2 <- lm(num_sp ~ latitude + elevation + NOISE, data = data)</pre>
```



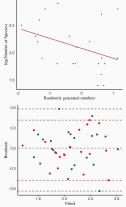
```
set.seed(84)
NOISE <- rnorm(22)
FIT2 <- lm(num_sp ~ latitude + elevation + NOISE, data = data)
summary(FIT2)
Call:
lm(formula = num_sp ~ latitude + elevation + NOISE, data = data)
Residuals:
     Min
                    Median
-0.47676 -0.09979 -0.03401 0.17771
                                    0.41594
Coefficients:
              Estimate Std. Error t value Pr(>t)
(Intercept) 10.9552493
                       2.2723853
                                  4.821 0.000137 ***
latitude
           -0.1986631 0.0531102 -3.741 0.001497 **
elevation
           -0.0014188 0.0003528 -4.021 0.000801 ***
NOTSE
            -0.1893679 0.0607058 -3.119 0.005922 **
Residual standard error: 0.2592 on 18 degrees of freedom
```

Multiple R-squared: 0.7179, Adjusted R-squared: 0.6708 F-statistic: 15.27 on 3 and 18 DF, p-value: 3.448e-05





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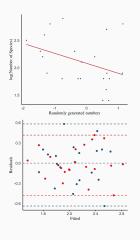


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-0.47676 -0.09979 -0.03401 0.17771
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(Intercept) 10.9552493
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Our previous R^2 was \sim 0.57, so on the surface it looks like we have a better model, even though we know for a fact it's just junk.





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Well, biological systems are complex, and variables can also interact with each other to influence outcomes (e.g., a breeze on a warm day is refreshing, a breeze in the middle of winter is not at all refreshing).

Interactions between explanatory variables are expressed as a product of the X's: $y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \varepsilon_i$

Parameter Interactions Cont.



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For a model with two predictor variables and their interaction,

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$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix}$$

Parameter Interactions Cont.



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$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix} \qquad \beta = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{pmatrix}$$

Parameter Interactions Cont.



For a model with two predictor variables and their interaction,

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{1i} x_{2i} + \varepsilon_i$$

$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix} \qquad \beta = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ \beta_3 \end{pmatrix} \qquad x = \begin{pmatrix} 1 & x_1 1 & x_2 1 & x_1 1 x_2 1 \\ 1 & x_1 2 & x_2 2 & x_1 2 x_2 2 \\ \vdots & \vdots & \vdots & \vdots \\ 1 & x_1 n & x_2 n & x_1 n x_2 n \end{pmatrix}$$

Parameter Interactions in Action





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It's conceivable that high elevation at high latitude is worse for ants than high elevation at lower latitudes.



Source: WallpaperAccess



Source: Wikipedia

Ant Richness: Analysis



Ant Richness: Analysis



We can easily model interaction terms using the : operator:

```
FIT <- lm(num_sp ~ latitude + elevation + latitude:elevation, data = data)
summary(FIT)</pre>
```

Ant Richness: Analysis



We can easily model interaction terms using the : operator:

```
FIT <- lm(num_sp ~ latitude + elevation + latitude:elevation, data = data)
summary (FIT)
Call:
lm(formula = num_sp ~ latitude * elevation, data = data)
Residuals:
     Min
             10 Median
                              30
                                       Max
-0.64627 -0.22755 0.07892 0.17200 0.60449
Coefficients:
                  Estimate Std. Error t value Pr(>t)
(Intercept)
                 12.2599423 5.1337948 2.388 0.0281 *
latitude
                 -0.2286208 0.1201511 -1.903 0.0732 .
elevation
                 -0.0064539 0.0190732 -0.338 0.7390
latitude: elevation 0.0001186 0.0004451 0.266 0.7929
Residual standard error: 0.3211 on 18 degrees of freedom
Multiple R-squared: 0.567, Adjusted R-squared: 0.4949
F-statistic: 7.858 on 3 and 18 DF, p-value: 0.001471
```

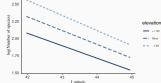
Ant Richness: Analysis



We can easily model interaction terms using the : operator:

FIT <- lm(num_sp ~ latitude + elevation + latitude:elevation, data = data)

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summary (FIT)
Call:
lm(formula = num sp ~ latitude * elevation, data = data)
Residuals:
     Min
                    Median
               10
                                  30
                                          Max
                                                                         2.25
-0.64627 -0.22755
                   0.07892 0.17200
                                                                        2.00
2.00
1.75
Coefficients:
                     Estimate Std. Error t value Pr(>t)
(Intercept)
                               5.1337948
                                            2.388
                                                     0.0281 *
                    12.2599423
latitude
                               0.1201511 -1.903
                                                     0.0732 .
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elevation
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                                                     0 7929
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In R we can include interaction terms in two ways:

FIT1 <- lm(num_sp ~ latitude:elevation, data = data)







```
FIT1 <- lm(num_sp ~ latitude:elevation, data = data)
Call.
lm(formula = num_sp ~ latitude:elevation, data = data)
Coefficients:
                  Estimate Std. Error t value Pr(>t)
(Intercept)
                  2.499e+00 1.402e-01 17.826 9.6e-14 ***
latitude:elevation -3.833e-05 1.152e-05 -3.328 0.00335 **
FIT2 <- lm(num_sp ~ latitude*elevation, data = data)
Call.
lm(formula = num_sp ~ latitude * elevation, data = data)
Coefficients:
                    Estimate Std. Error t value Pr(>t)
(Intercept)
                 12.2599423 5.1337948 2.388 0.0281 *
latitude
                  -0.2286208 0.1201511 -1.903 0.0732 .
elevation
                  -0.0064539 0.0190732 -0.338 0.7390
latitude:elevation 0.0001186 0.0004451 0.266 0.7929
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Figure out what each model means given the process you are modeling and whether a model with or without the main effects makes more sense given your theory or hypothesis.



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...and always use objective measures to decide on what parameters should be included/discarded.