

Unfolding_curves

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$$\min + \frac{1-\min}{(1+e^{(-\text{slope}(T_m-T_{emp}))})}$$

Loading libraries and functions

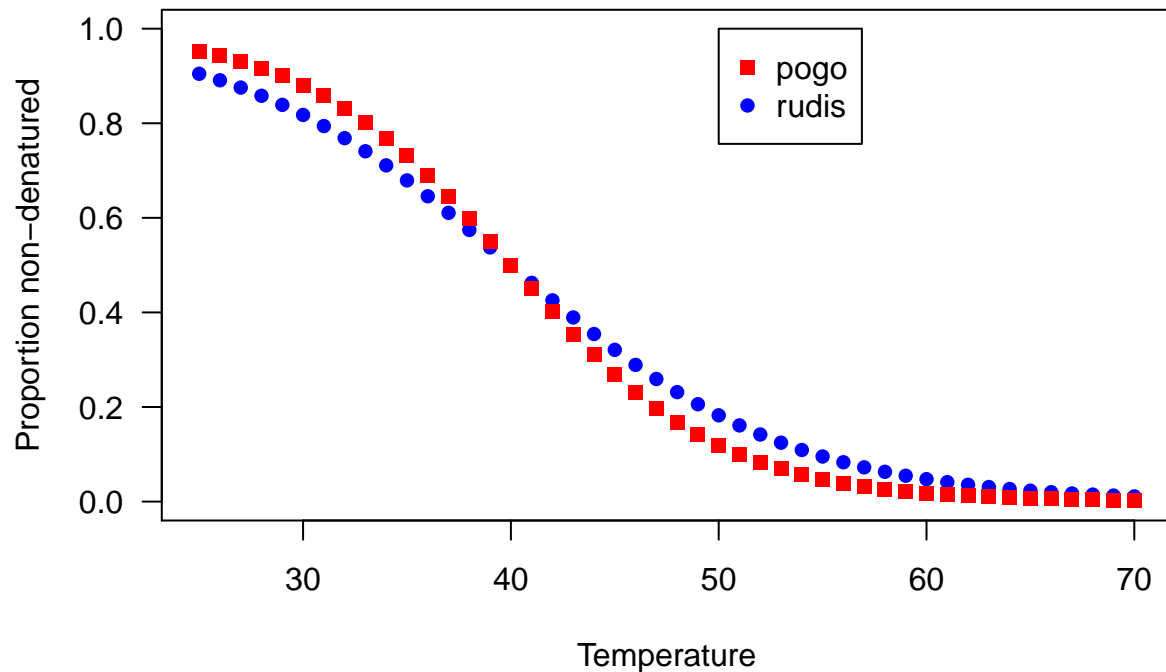
```
library(plyr)
library(ggplot2)
library(tidyr)
library(minpack.lm)

nls.fit<-function(data=data){
  y<-nlsLM(unfolding ~ min+ (1-min)/(1+exp((-slope*(Tm-T)))),data=data,
           start=list(slope=.5,Tm=45,min=.3),
           trace=TRUE,control=nls.control(warnOnly = TRUE, tol = 1e-05, maxiter=1000))
  #return(y)
  return(summary(y)$coefficients)
}
```

#Function that predicts values given parameter estimates of curves

```
fud<-function(T=seq(25,70,1),Tm=40,slope=.5,max=1,min=0){
  y<-min+ (max-min)/(1+exp((-slope*(Tm-T))))
  return(y)
}
```

```
plot(seq(25,70,1),fud(slope=.15),col="blue",pch=16,las=1,ylim=c(0,1),ylab="Proportion non-denatured",xlab="Temperature")
points(seq(25,70,1),fud(slope=.2),col="red",pch=15)#pogo
legend(50,1,c("pogo","rudis"),pch=c(15,16),col=c("red","blue"))
```



20160520

```
x<-read.csv("../Data/20160520_total_protein_unfolding.csv")
x.par<-subset(x,x$TN=="N" & x$Species!="A. picea")

str(x.par)
```

```
## 'data.frame': 72 obs. of 9 variables:
## $ Date : int 20160407 20160407 20160407 20160407 20160407 20160407 20160407 20160407 20160407 20160407 ...
## $ Replicate: int 1 1 1 1 1 1 1 1 1 1 ...
## $ Species : Factor w/ 3 levels "A. picea","A. rudis",...: 3 3 3 3 3 3 3 3 3 3 ...
## $ Colony : Factor w/ 8 levels "AVON","CENT WOODS",...: 5 5 5 5 5 5 5 5 5 5 ...
## $ TN : Factor w/ 2 levels "N","T": 1 1 1 1 1 1 1 1 1 1 ...
## $ Sample : int 1 2 3 4 5 6 7 8 9 10 ...
## $ T : int 25 30 35 40 43 45 48 50 55 60 ...
## $ prot_conc: num 0.886 0.874 0.865 0.748 0.714 ...
## $ unfolding: num 1 0.986 0.976 0.843 0.805 ...
```

```
x.par
```

```
##      Date Replicate Species Colony TN Sample T prot_conc
## 13 20160407      1 P. barbatus WWRQ-45 N      1 25 0.8864270
## 14 20160407      1 P. barbatus WWRQ-45 N      2 30 0.8740756
## 15 20160407      1 P. barbatus WWRQ-45 N      3 35 0.8651183
## 16 20160407      1 P. barbatus WWRQ-45 N      4 40 0.7475508
## 17 20160407      1 P. barbatus WWRQ-45 N      5 43 0.7137178
## 18 20160407      1 P. barbatus WWRQ-45 N      6 45 0.6491979
## 19 20160407      1 P. barbatus WWRQ-45 N      7 48 0.5503542
```

## 20	20160407	1	P. barbatus	WWRQ-45	N	8	50	0.5312472
## 21	20160407	1	P. barbatus	WWRQ-45	N	9	55	0.4355170
## 22	20160407	1	P. barbatus	WWRQ-45	N	10	60	0.3633674
## 23	20160407	1	P. barbatus	WWRQ-45	N	11	65	0.3690008
## 24	20160407	1	P. barbatus	WWRQ-45	N	12	70	0.3648026
## 61	20160419	2	P. barbatus	WWRQ-53	N	1	25	0.7578268
## 62	20160419	2	P. barbatus	WWRQ-53	N	2	30	0.7842361
## 63	20160419	2	P. barbatus	WWRQ-53	N	3	35	0.7450706
## 64	20160419	2	P. barbatus	WWRQ-53	N	4	40	0.6982019
## 65	20160419	2	P. barbatus	WWRQ-53	N	5	43	0.6317106
## 66	20160419	2	P. barbatus	WWRQ-53	N	6	45	0.5788304
## 67	20160419	2	P. barbatus	WWRQ-53	N	7	48	0.5414056
## 68	20160419	2	P. barbatus	WWRQ-53	N	8	50	0.4824624
## 69	20160419	2	P. barbatus	WWRQ-53	N	9	55	0.4367095
## 70	20160419	2	P. barbatus	WWRQ-53	N	10	60	0.3377081
## 71	20160419	2	P. barbatus	WWRQ-53	N	11	65	0.3350468
## 72	20160419	2	P. barbatus	WWRQ-53	N	12	70	0.3212022
## 109	20160517	3	P. barbatus	WWRQ-8	N	1	25	0.8370028
## 110	20160517	3	P. barbatus	WWRQ-8	N	2	30	0.9021145
## 111	20160517	3	P. barbatus	WWRQ-8	N	3	35	0.8736166
## 112	20160517	3	P. barbatus	WWRQ-8	N	4	40	0.7886599
## 113	20160517	3	P. barbatus	WWRQ-8	N	5	43	0.6950219
## 114	20160517	3	P. barbatus	WWRQ-8	N	6	45	0.6397358
## 115	20160517	3	P. barbatus	WWRQ-8	N	7	48	0.5838863
## 116	20160517	3	P. barbatus	WWRQ-8	N	8	50	0.5426064
## 117	20160517	3	P. barbatus	WWRQ-8	N	9	55	0.4657911
## 118	20160517	3	P. barbatus	WWRQ-8	N	10	60	0.4151872
## 119	20160517	3	P. barbatus	WWRQ-8	N	11	65	0.3977230
## 120	20160517	3	P. barbatus	WWRQ-8	N	12	70	0.3761968
## 133	20160505	1	A. rudis	Duke 1	N	1	25	0.8078769
## 134	20160505	1	A. rudis	Duke 1	N	2	30	0.8028302
## 135	20160505	1	A. rudis	Duke 1	N	3	35	0.7205932
## 136	20160505	1	A. rudis	Duke 1	N	4	40	0.7019431
## 137	20160505	1	A. rudis	Duke 1	N	5	43	0.6528357
## 138	20160505	1	A. rudis	Duke 1	N	6	45	0.5831983
## 139	20160505	1	A. rudis	Duke 1	N	7	48	0.5405197
## 140	20160505	1	A. rudis	Duke 1	N	8	50	0.4542220
## 141	20160505	1	A. rudis	Duke 1	N	9	55	0.4383184
## 142	20160505	1	A. rudis	Duke 1	N	10	60	0.3755274
## 143	20160505	1	A. rudis	Duke 1	N	11	65	0.3190845
## 144	20160505	1	A. rudis	Duke 1	N	12	70	0.2877233
## 157	20160510	2	A. rudis	Yates 2	N	1	25	0.8004311
## 158	20160510	2	A. rudis	Yates 2	N	2	30	0.7319170
## 159	20160510	2	A. rudis	Yates 2	N	3	35	0.7828180
## 160	20160510	2	A. rudis	Yates 2	N	4	40	0.7139815
## 161	20160510	2	A. rudis	Yates 2	N	5	43	0.6314721
## 162	20160510	2	A. rudis	Yates 2	N	6	45	0.5693688
## 163	20160510	2	A. rudis	Yates 2	N	7	48	0.5390834
## 164	20160510	2	A. rudis	Yates 2	N	8	50	0.5156809
## 165	20160510	2	A. rudis	Yates 2	N	9	55	0.4284167
## 166	20160510	2	A. rudis	Yates 2	N	10	60	0.3607075
## 167	20160510	2	A. rudis	Yates 2	N	11	65	0.3393937
## 168	20160510	2	A. rudis	Yates 2	N	12	70	0.2866893
## 181	20160519	3	A. rudis	Lex 13	N	1	25	0.7767705

## 182	20160519	3	A. rudis	Lex 13	N	2 30	0.7506128
## 183	20160519	3	A. rudis	Lex 13	N	3 35	0.7074833
## 184	20160519	3	A. rudis	Lex 13	N	4 40	0.6701137
## 185	20160519	3	A. rudis	Lex 13	N	5 43	0.6007297
## 186	20160519	3	A. rudis	Lex 13	N	6 45	0.5416209
## 187	20160519	3	A. rudis	Lex 13	N	7 48	0.4736794
## 188	20160519	3	A. rudis	Lex 13	N	8 50	0.4595478
## 189	20160519	3	A. rudis	Lex 13	N	9 55	0.4089731
## 190	20160519	3	A. rudis	Lex 13	N	10 60	0.3148353
## 191	20160519	3	A. rudis	Lex 13	N	11 65	0.2149658
## 192	20160519	3	A. rudis	Lex 13	N	12 70	0.2023652
##	unfolding						
## 13	1.0000000						
## 14	0.9860661						
## 15	0.9759611						
## 16	0.8433303						
## 17	0.8051625						
## 18	0.7323761						
## 19	0.6208680						
## 20	0.5993130						
## 21	0.4913174						
## 22	0.4099236						
## 23	0.4162788						
## 24	0.4115428						
## 61	0.9663248						
## 62	1.0000000						
## 63	0.9500591						
## 64	0.8902956						
## 65	0.8055107						
## 66	0.7380818						
## 67	0.6903605						
## 68	0.6152004						
## 69	0.5568598						
## 70	0.4306205						
## 71	0.4272270						
## 72	0.4095733						
## 109	0.9278232						
## 110	1.0000000						
## 111	0.9684099						
## 112	0.8742349						
## 113	0.7704365						
## 114	0.7091515						
## 115	0.6472419						
## 116	0.6014829						
## 117	0.5163325						
## 118	0.4602378						
## 119	0.4408787						
## 120	0.4170167						
## 133	1.0000000						
## 134	0.9937532						
## 135	0.8919591						
## 136	0.8688739						
## 137	0.8080881						
## 138	0.7218901						

```
## 139 0.6690620
## 140 0.5622416
## 141 0.5425559
## 142 0.4648325
## 143 0.3949667
## 144 0.3561475
## 157 1.0000000
## 158 0.9144036
## 159 0.9779956
## 160 0.8919962
## 161 0.7889150
## 162 0.7113277
## 163 0.6734913
## 164 0.6442540
## 165 0.5352325
## 166 0.4506416
## 167 0.4240137
## 168 0.3581686
## 181 1.0000000
## 182 0.9663251
## 183 0.9108010
## 184 0.8626920
## 185 0.7733683
## 186 0.6972728
## 187 0.6098062
## 188 0.5916134
## 189 0.5265044
## 190 0.4053132
## 191 0.2767430
## 192 0.2605213
```

```
mod1<-ddply(x.par,.(Species,Colony),nls.fit)
```

```
## It.    0, RSS =    0.219773, Par. =          0.5          45          0.3
## It.    1, RSS =    0.0260954, Par. =    0.142137    45.3563    0.431124
## It.    2, RSS =    0.00930654, Par. =     0.16001    47.7898    0.361913
## It.    3, RSS =    0.00864097, Par. =    0.159122    47.3355    0.362318
## It.    4, RSS =    0.00863626, Par. =    0.160502     47.3    0.363555
## It.    5, RSS =    0.0086362, Par. =    0.160607     47.293    0.363733
## It.    6, RSS =    0.00863619, Par. =    0.160626    47.2922    0.363758
## It.    7, RSS =    0.00863619, Par. =    0.160628    47.292    0.363762
## It.    0, RSS =    0.171415, Par. =          0.5          45          0.3
## It.    1, RSS =    0.0884104, Par. =    0.352574    45.2134    0.327346
## It.    2, RSS =    0.0807163, Par. =    0.0962454    46.0299    0.337383
## It.    3, RSS =    0.0392659, Par. =     0.1224    46.1273    0.325633
## It.    4, RSS =    0.0143309, Par. =    0.154489    47.439    0.296735
## It.    5, RSS =    0.00989232, Par. =    0.134166    49.2347    0.235338
## It.    6, RSS =    0.00944728, Par. =    0.134368    49.6916    0.218841
## It.    7, RSS =    0.00944301, Par. =    0.133369    49.7538    0.216266
## It.    8, RSS =    0.00944293, Par. =    0.133407    49.7584    0.216165
## It.    9, RSS =    0.00944293, Par. =    0.133389    49.7593    0.216129
## It.   10, RSS =    0.00944293, Par. =     0.13339    49.7594    0.216128
## It.    0, RSS =    0.254525, Par. =          0.5          45          0.3
## It.    1, RSS =    0.0645737, Par. =    0.102651    45.3114    0.443002
```

```
## It. 2, RSS = 0.0195806, Par. = 0.140332 50.5569 0.336411
## It. 3, RSS = 0.0101722, Par. = 0.150187 48.0116 0.362697
## It. 4, RSS = 0.00993881, Par. = 0.157089 48.0061 0.363389
## It. 5, RSS = 0.00993836, Par. = 0.1573 47.9873 0.363714
## It. 6, RSS = 0.00993835, Par. = 0.157342 47.9852 0.363774
## It. 7, RSS = 0.00993835, Par. = 0.157347 47.985 0.363781
## It. 0, RSS = 0.188833, Par. = 0.5 45 0.3
## It. 1, RSS = 0.0185444, Par. = 0.15723 45.286 0.428524
## It. 2, RSS = 0.00328565, Par. = 0.210424 46.0744 0.408249
## It. 3, RSS = 0.00299925, Par. = 0.214541 45.9965 0.403356
## It. 4, RSS = 0.00299914, Par. = 0.214231 45.9988 0.403236
## It. 5, RSS = 0.00299914, Par. = 0.21426 45.9988 0.403245
## It. 6, RSS = 0.00299914, Par. = 0.214257 45.9988 0.403244
## It. 0, RSS = 0.260606, Par. = 0.5 45 0.3
## It. 1, RSS = 0.0334231, Par. = 0.129349 45.4537 0.452191
## It. 2, RSS = 0.00630438, Par. = 0.171619 48.2124 0.394745
## It. 3, RSS = 0.00437035, Par. = 0.180754 47.273 0.40141
## It. 4, RSS = 0.00436132, Par. = 0.182459 47.2859 0.401357
## It. 5, RSS = 0.00436129, Par. = 0.182341 47.2859 0.401311
## It. 6, RSS = 0.00436129, Par. = 0.182349 47.2859 0.401312
## It. 7, RSS = 0.00436129, Par. = 0.182348 47.2859 0.401312
## It. 0, RSS = 0.230184, Par. = 0.5 45 0.3
## It. 1, RSS = 0.0180401, Par. = 0.158044 44.9351 0.455391
## It. 2, RSS = 0.00704717, Par. = 0.194938 45.8341 0.425468
## It. 3, RSS = 0.00683008, Par. = 0.201473 45.5853 0.427459
## It. 4, RSS = 0.00682798, Par. = 0.202695 45.5701 0.427996
## It. 5, RSS = 0.00682795, Par. = 0.202804 45.5669 0.42808
## It. 6, RSS = 0.00682795, Par. = 0.202819 45.5665 0.42809
## It. 7, RSS = 0.00682795, Par. = 0.202821 45.5665 0.428092
```

```
mod1$parameter<-rep(c("slope", "Tm", "min"), length(mod1$Species)/3)
knitr::kable(mod1)
```

Species	Colony	Estimate	Std. Error	t value	Pr(> t)	parameter
A. rudis	Duke 1	0.1606280	0.0206403	7.782238	0.0000276	slope
A. rudis	Duke 1	47.2920297	0.9451544	50.036301	0.0000000	Tm
A. rudis	Duke 1	0.3637620	0.0293990	12.373285	0.0000006	min
A. rudis	Lex 13	0.1333902	0.0159832	8.345673	0.0000158	slope
A. rudis	Lex 13	49.7593929	1.2760137	38.995972	0.0000000	Tm
A. rudis	Lex 13	0.2161279	0.0451703	4.784737	0.0009947	min
A. rudis	Yates 2	0.1573466	0.0220329	7.141430	0.0000542	slope
A. rudis	Yates 2	47.9849648	1.0899761	44.023870	0.0000000	Tm
A. rudis	Yates 2	0.3637813	0.0336777	10.801853	0.0000019	min
P. barbatus	WWRQ-45	0.2142567	0.0165774	12.924625	0.0000004	slope
P. barbatus	WWRQ-45	45.9987927	0.3837543	119.865208	0.0000000	Tm
P. barbatus	WWRQ-45	0.4032438	0.0126671	31.834069	0.0000000	min
P. barbatus	WWRQ-53	0.1823480	0.0173963	10.482009	0.0000024	slope
P. barbatus	WWRQ-53	47.2858982	0.5958843	79.354167	0.0000000	Tm
P. barbatus	WWRQ-53	0.4013122	0.0184886	21.705927	0.0000000	min
P. barbatus	WWRQ-8	0.2028211	0.0245990	8.245113	0.0000174	slope
P. barbatus	WWRQ-8	45.5664742	0.6340253	71.868543	0.0000000	Tm
P. barbatus	WWRQ-8	0.4280916	0.0194756	21.980921	0.0000000	min

```
Tm<-subset(mod1,mod1$parameter=="Tm")
Tm
```

```
##      Species Colony Estimate Std. Error  t value    Pr(>|t|)
## 2    A. rudis Duke 1 47.29203  0.9451544  50.03630 2.552282e-12
## 5    A. rudis Lex 13 49.75939  1.2760137  38.99597 2.383465e-11
## 8    A. rudis Yates 2 47.98496  1.0899761  44.02387 8.043506e-12
## 11 P. barbatus WWRQ-45 45.99879  0.3837543 119.86521 9.943058e-16
## 14 P. barbatus WWRQ-53 47.28590  0.5958843  79.35417 4.057007e-14
## 17 P. barbatus WWRQ-8 45.56647  0.6340253  71.86854 9.883895e-14
##      parameter
## 2          Tm
## 5          Tm
## 8          Tm
## 11         Tm
## 14         Tm
## 17         Tm
```

```
summary(aov(Estimate~Species,data=Tm))
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Species      1  6.376   6.376    5.271 0.0833 .
## Residuals    4  4.839   1.210
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

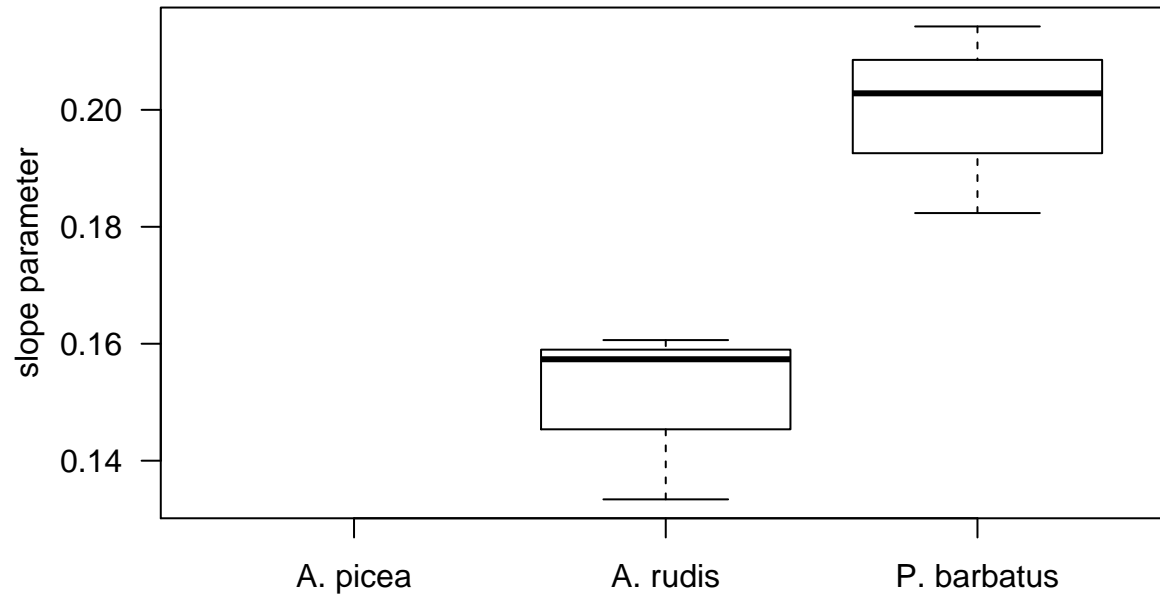
```
slope<-subset(mod1,mod1$parameter=="slope")
slope
```

```
##      Species Colony Estimate Std. Error  t value    Pr(>|t|)
## 1    A. rudis Duke 1 0.1606280 0.02064034  7.782238 2.757621e-05
## 4    A. rudis Lex 13 0.1333902 0.01598316  8.345673 1.576248e-05
## 7    A. rudis Yates 2 0.1573466 0.02203293  7.141430 5.416624e-05
## 10 P. barbatus WWRQ-45 0.2142567 0.01657740 12.924625 4.081714e-07
## 13 P. barbatus WWRQ-53 0.1823480 0.01739629 10.482009 2.414984e-06
## 16 P. barbatus WWRQ-8 0.2028211 0.02459895  8.245113 1.737865e-05
##      parameter
## 1      slope
## 4      slope
## 7      slope
## 10     slope
## 13     slope
## 16     slope
```

```
summary(aov(Estimate~Species,data=slope))
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Species      1 0.003654 0.003654   15.15 0.0177 *
## Residuals    4 0.000965 0.000241
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
boxplot(Estimate~Species,data=slope,las=1,ylab="slope parameter")
```



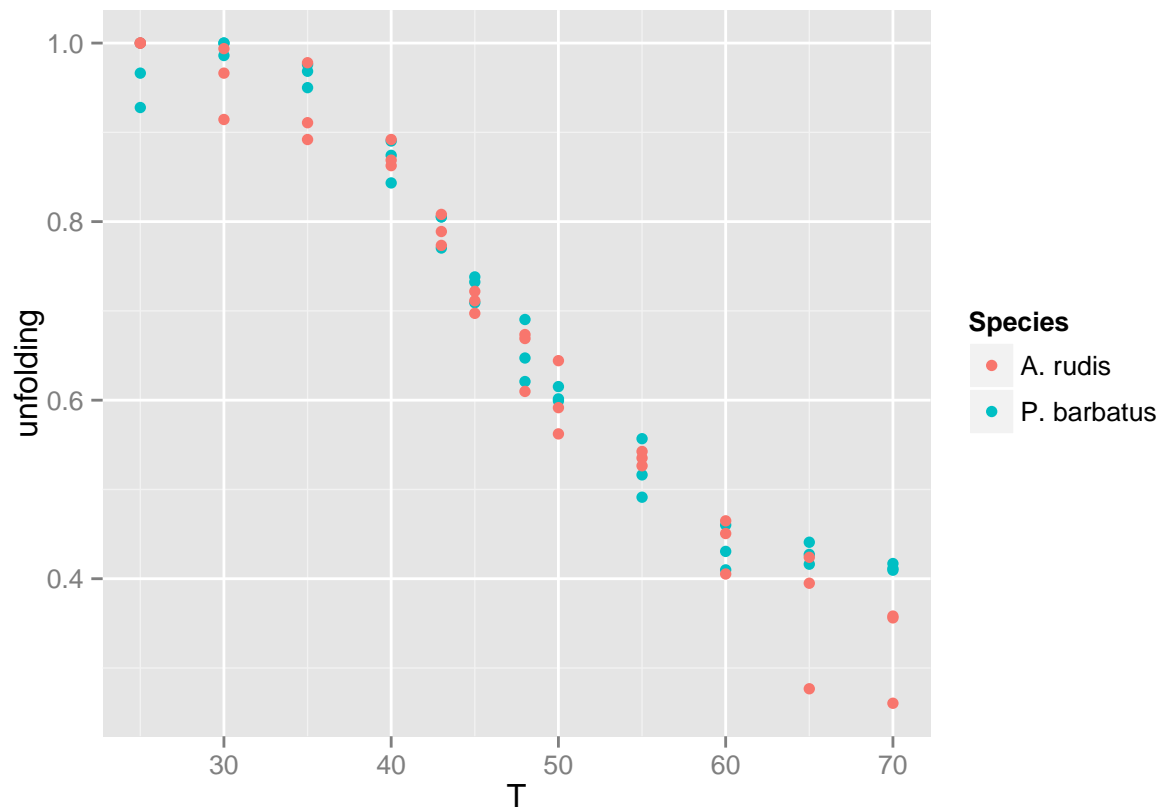
```
min<-subset(mod1,mod1$parameter=="min")
min
```

```
##      Species Colony Estimate Std. Error  t value    Pr(>|t|)
## 3    A. rudis  Duke 1 0.3637620 0.02939898 12.373285 5.928939e-07
## 6    A. rudis  Lex 13 0.2161279 0.04517028  4.784737 9.946745e-04
## 9    A. rudis  Yates 2 0.3637813 0.03367767 10.801853 1.876309e-06
## 12   P. barbatus WWRQ-45 0.4032438 0.01266705 31.834069 1.462621e-10
## 15   P. barbatus WWRQ-53 0.4013122 0.01848860 21.705926 4.405987e-09
## 18   P. barbatus WWRQ-8 0.4280916 0.01947560 21.980921 3.941519e-09
##      parameter
## 3          min
## 6          min
## 9          min
## 12         min
## 15         min
## 18         min
```

```
summary(aov(Estimate~Species,data=min))
```

```
##      Df Sum Sq Mean Sq F value Pr(>F)
## Species    1 0.01392 0.013918   3.717  0.126
## Residuals   4 0.01498 0.003745
```

```
ggplot(x.par,aes(x=T,y=unfolding,colour=Species))+geom_point()
```

Comparing aphaeno and pogo

```
#pogo
T<-c(25,30,35,40,43,45,48,50,55,60,65,70)
ten<-c(1.00,0.984,0.974,0.795,0.787,0.726,0.640,0.574,0.482,0.379,0.387,0.392)
ten.dat<-as.data.frame(cbind(T,ten));names(ten.dat)[2]<-"unfolding"
unfold_10min<-nls.fit(ten.dat)
#Aphaeno
avon<-c(0.963,1.00,0.940,0.882,0.755,0.627,0.600,0.549,0.502,0.444,0.368,0.379)
aph<-as.data.frame(cbind(T,avon));names(aph)[2]<-"unfolding"
aph.fit<-nls.fit(aph)
#Table of fitted curves
knitr::kable(round(cbind(unfold_10min,aph.fit),3))
#difference in TMs
46.16-44.754

#lets plot these out with all parameters
plot(seq(25,70,.1),fud(T=seq(25,70,.1),max=1,min=0.3958177,Tm=44.7544257,slope=0.2156598),pch=16,col="blue",lwd=3)
points(seq(25,70,.1),fud(T=seq(25,70,.1),max=1,min=0.3704093,Tm=46.159662,slope=0.1923073),pch=16,col="red",lwd=3)
points(ten.dat$T,ten.dat$unfolding,pch=16,col="red",cex=2)
points(aph$T,aph$unfolding,pch=16,col="blue",cex=2)
legend(55,1,c("Pogonomyrmex barbatus","Aphaenogaster picea"),text.font=3,pch=16,col=c("red","blue"))
#abline(v=c(46.1596629,44.7544257),lty=c(1,3),lwd=3)
```

```
##plot by only slope
plot(seq(25,70,.1),fud(T=seq(25,70,.1),slope= 0.192,Tm=45),pch=16,col="blue",ylab="Proportion non-denatured")
points(seq(25,70,.1),fud(T=seq(25,70,.1),slope= 0.216,Tm=45),pch=16,col="red")
legend(55,1,c("Pogonomyrmex barbatus","Aphaenogaster picea"),text.font=3,pch=16,col=c("red","blue"))
```

comparing 10 vs 20 min

```
# 10 min inc
#earlier
unfold_10min<-nls.fit(ten.dat)
# 20 min incubation
twen<-c(0.977,0.988,1.00,0.822,0.664,0.564,0.479,0.473,0.432,0.350,0.315,0.304)
ty.dat<-as.data.frame(cbind(T,twen));names(ty.dat)[2]<-"unfolding"
unfold_20min<-nls.fit(ty.dat);unfold_20min

knitr::kable(round(cbind(unfold_10min,unfold_20min),3))

#plots

plot(seq(25,70,.1),fud(T=seq(25,70,.1),max=1,min=0.3452337,Tm=43.2788185,slope=0.2824321),pch=16,col="red",lwd=2)
points(seq(25,70,.1),fud(T=seq(25,70,.1),max=1,min=0.3704093,Tm=46.159662,slope=0.1923073),pch=16,col="black",lwd=2)
points(ten.dat$T,ten.dat$unfolding,pch=16,col="black",cex=2)
points(ty.dat$T,ty.dat$unfolding,pch=16,col="red",cex=2)
abline(v=c(46.1596629,43.2788185),lty=c(1,3),lwd=3)
legend(55,1,c("10 minute","20 minute"),pch=16,col=c("black","red"))
```

How to sample a reaction norm?

```
unfolding<-fud(T=seq(25,70,1),Tm=45,slope=.2,min=.4)
T<-seq(25,70,1)
com<-cbind(T,unfolding);head(com)
plot(T,unfolding,ylab="Proportion non-denatured",xlab="Temperature (C)",las=1)

#sample random points
n<-as.data.frame(com[sample(nrow(com), 10), ])# randomly sampling
n<-n[order(n$T),]
n$unfolding<-n[,2]+rnorm(mean=0,sd=0.02,n=10)# adding error

plot(n$T,n$unfolding,pch=16,col="black",cex=2,ylim=c(.4,1))
lines(T,unfolding)
tt<-nls.fit(n)
tt
lines(seq(25,70,.1),fud(Tm=tt[2],slope=tt[1],min=tt[3],T=seq(25,70,.1)),col="red",lwd=2)
legend(45,1,c("Known curve","Modeled based on points"),pch="-",col=c("black","red"),cex=1.5)
knitr::kable(tt)
#mean(nndist(n$T))
```

```
#try nearest neighbor  
#http://www.statsoft.com/textbook/k-nearest-neighbors  
library(spatstat)
```