Analyses Cerastium

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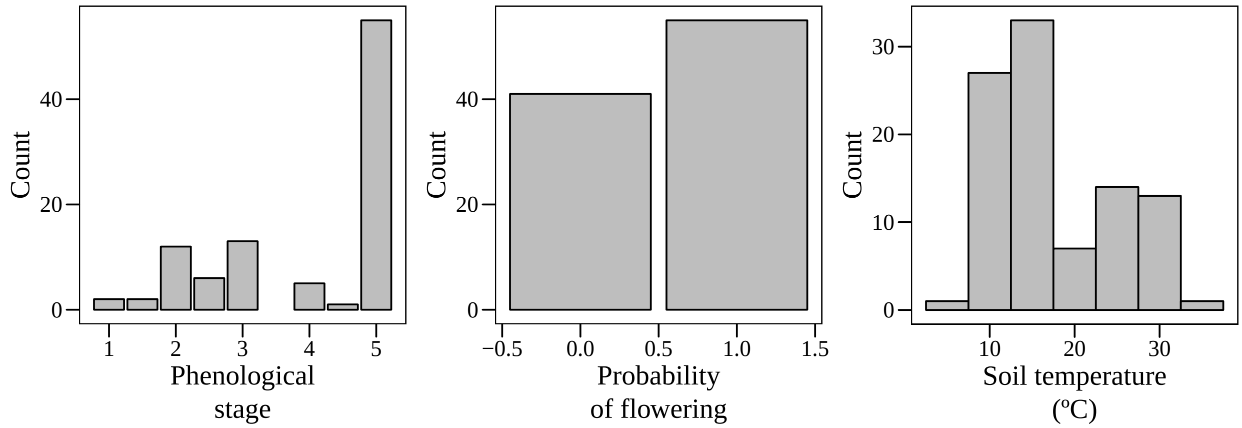
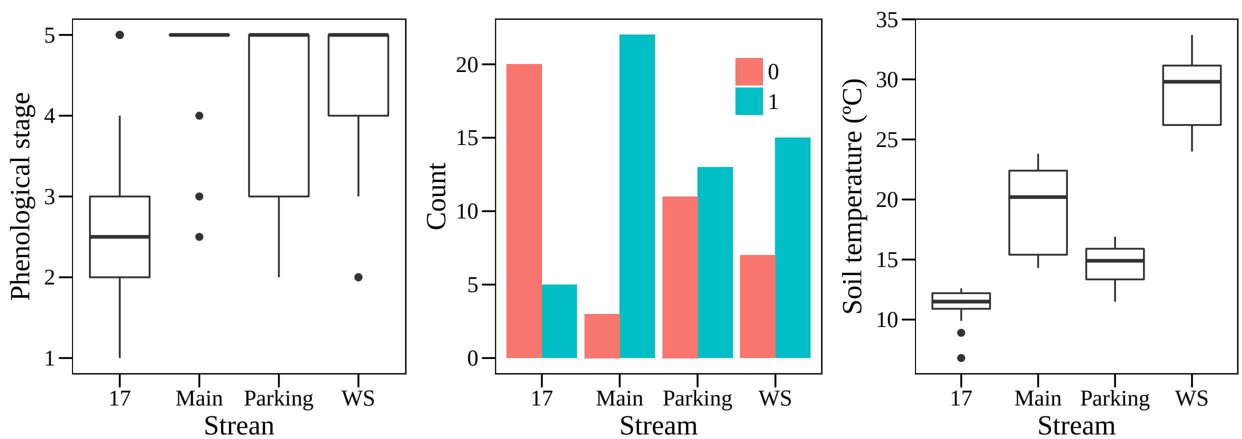
Field data 2015: Distributions



Field data 2015: Differences among streams



Differences among streams in phenological stage: Anova and Tukey HSD

```
kable(prettify(with(data_f_2015,Anova(lm(stage~stream)))))
```

	Sum Sq	Df	F value	Pr(>F)	
stream	53.6888	3	15.4542	<0.001	***

```
kable(prettify(as.data.frame(with(data_f_2015,TukeyHSD(aov(lm(stage~stream))))$stream)))
```

	diff	lwr	upr	p adj
Main-17	1.9600000	1.1635790	2.756421	0.0000000
Parking-17	1.1591667	0.3544924	1.963841	0.0016202
WS-17	1.5890909	0.7659668	2.412215	0.0000130
Parking-Main	-0.8008333	-1.6055076	0.003841	0.0515857
WS-Main	-0.3709091	-1.1940332	0.452215	0.6415302
WS-Parking	0.4299242	-0.4011880	1.261036	0.5315756

Differences among streams in proability of flowering: Anova and Tukey HSD

```
kable(prettify(with(data_f_2015,Anova(glm(flowered~stream,family="binomial")))))
```

	LR Chisq	Df	Pr(>Chisq)	
stream	27.0431	3	<0.001	***

```
kable(prettify(as.data.frame(with(data_f_2015,TukeyHSD(aov(as.numeric(flowered)~stream)))$stream)))
```

	diff	lwr	upr	p adj
Main-17	0.6800000	0.3584089	1.0015911	0.0000017
Parking-17	0.3416667	0.0167429	0.6665905	0.0353782
WS-17	0.4818182	0.1494445	0.8141919	0.0014942
Parking-Main	-0.3383333	-0.6632571	-0.0134095	0.0379492
WS-Main	-0.1981818	-0.5305555	0.1341919	0.4063784
WS-Parking	0.1401515	-0.1954478	0.4757508	0.6948481

Differences among streams in temperature: Anova and Tukey HSD

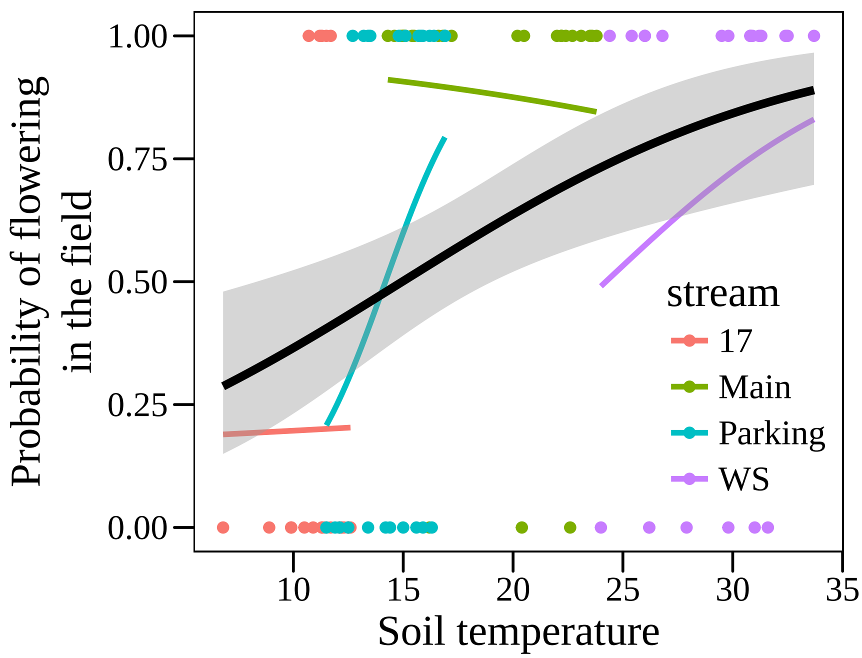
```
kable(prettify(with(data_f_2015,Anova(lm(temp~stream)))))
```

	Sum Sq	Df	F value	Pr(>F)	
stream	4110.748	3	213.7775	<0.001	***

```
kable(prettify(as.data.frame(with(data_f_2015,TukeyHSD(aov(lm(temp~stream))))$stream)))
```

	diff	lwr	upr	p adj
Main-17	7.772000	5.898285	9.645715	0.00e+00
Parking-17	3.294167	1.401034	5.187299	9.42e-05
WS-17	17.749091	15.812552	19.685630	0.00e+00
Parking-Main	-4.477833	-6.370966	-2.584701	1.00e-07
WS-Main	9.977091	8.040552	11.913630	0.00e+00
WS-Parking	14.454924	12.499592	16.410256	0.00e+00

Hypothesis 1: Local soil warming leads to an earlier phenology (2015 and 2017)



Logistic regression (relationship among probability of flowering and soil temperature) with pooled data

```
kable(prettify(summary(glm(flowered~temp,data=data_f_2015,family="binomial"))))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)	
(Intercept)	-1.6666857	0.188872	0.0504618	0.6399272	0.6433108	-2.590794	0.01	**
temp	0.1114387	1.117885	1.0462921	1.2057695	0.0358129	3.111692	0.002	**

There is a strong overall relatiohsnip among probability of flowering and soil temperature.

Logistic regression for each stream

```
kable(prettify(summary(glm(flowered~temp,data=subset(data_f_2015,stream=="17"),family="binomial"))))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.5583006	0.2104935	0.0000026	575.210838	4.4573377	-0.3496034	0.727
temp	0.0152656	1.0153827	0.4982892	2.673228	0.3928292	0.0388606	0.969

```
kable(prettify(summary(glm(flowered~temp,data=subset(data_f_2015,stream=="Main"),family="binomial"))))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)
(Intercept)	3.2628934	26.1250173	0.0375709	1.409812e+05	3.5647263	0.9153279	0.36
temp	-0.0657001	0.9364116	0.6258017	1.337007e+00	0.1787638	-0.3675247	0.713

```
kable(prettify(summary(glm(flowered~temp,data=subset(data_f_2015,stream=="Parking"),family="binomial"))))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)	
(Intercept)	-7.0604560	0.0008584	0.0000001	1.763186	4.1609369	-1.696843	0.09	.
temp	0.4975863	1.6447466	0.9758600	3.072677	0.2849575	1.746177	0.081	.

```
kable(prettify(summary(glm(flowered~temp,data=subset(data_f_2015,stream=="WS"),family="binomial"))))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)
(Intercept)	-4.0550074	0.0173354	0.0000009	187.805270	4.7582873	-0.852199	0.394
temp	0.1674113	1.1822404	0.8594305	1.678054	0.1658428	1.009457	0.313

Within each stream, there are no significant relationships.

Logistic regression including temperature and stream

```
# Different slopes and intercepts for each stream
model1<-glm(flowered~temp*stream,family="binomial",data_f_2015)
kable(prettify(summary(model1)))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)
(Intercept)	-1.5583006	0.2104935	0.0000026	5.752108e+02	4.4573377	-0.3496034	0.727
temp	0.0152656	1.0153827	0.4982892	2.673228e+00	0.3928292	0.0388606	0.969
stream: Main	4.8211939	124.1131851	0.0041193	7.178108e+07	5.7074629	0.8447175	0.398
stream: Parking	-5.5021554	0.0040780	0.0000000	2.579510e+03	6.0976434	-0.9023413	0.367
stream: WS	-2.4967069	0.0823558	0.0000003	1.113649e+05	6.5199047	-0.3829361	0.702
temp:streamMain	-0.0809657	0.9222253	0.3321141	2.033585e+00	0.4315916	-0.1875980	0.851
temp:streamParking	0.4823207	1.6198292	0.5466614	4.107605e+00	0.4852995	0.9938620	0.32
temp:streamWS	0.1521457	1.1643298	0.4237360	2.553313e+00	0.4264020	0.3568128	0.721

```
# Common slope, different intercepts for each stream
model2<-glm(flowered~temp+stream,family="binomial",data_f_2015)
kable(prettify(summary(model2)))
```

	Estimate	Odds Ratio	CI (lower)	CI (upper)	Std. Error	z value	Pr(>|z|)	
(Intercept)	-2.8302954	0.0589954	0.0038896	0.7220361	1.3169346	-2.1491540	0.032	*
temp	0.1275365	1.1360263	0.9252548	1.4162684	0.1069991	1.1919398	0.233	
stream: Main	2.4703883	11.8270379	1.6592861	111.0947792	1.0579993	2.3349621	0.02	*
stream: Parking	1.1427432	3.1353575	0.7695188	13.9338737	0.7307727	1.5637464	0.118	
stream: WS	-0.0841986	0.9192486	0.0174175	43.9307406	1.9701665	-0.0427368	0.966	

```r
anova(model1,model2, test="Chisq") # Likelihood ratio test comparing both models
```

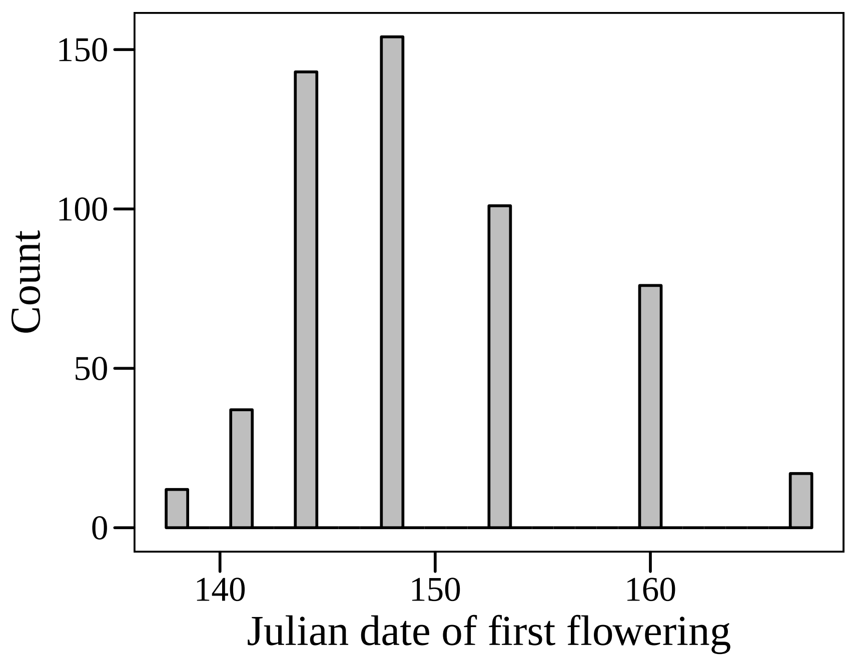
```
## Analysis of Deviance Table
##
## Model 1: flowered ~ temp * stream
## Model 2: flowered ~ temp + stream
##   Resid. Df Resid. Dev Df Deviance Pr(>Chi)
## 1        88     99.323
## 2        91    102.522 -3  -3.1996   0.3619
```

There is no support for significant differences between slopes (despite the graph!), so we keep model2 with a common slope and different intercepts for each stream. We cannot really separate the effects of streams from the effects of temperature, as streams have very different ranges of temperatures, i.e. effects of streams are likely to be mostly effects of temperatures. This means that differences in temperature at larger scales (among streams) are more important than differences at small scales (within streams).

Hypothesis 2: Phenotypic selection for early flowering is stronger in colder sites with shorter growing seasons (2017)

Hypothesis 3: As a consequence of selection, differences in phenology in a common environment are related to soil temperature at the plant origin in a counter-gradient fashion, with plants originating from colder sites flowering earlier

Histogram of Julian date of first flowering in the common garden (2017)



Effect of mother plant (and stream) on the flowering phenology in the common garden

Mother plant is treated as a random effect (we are not interested in the effect of "a particular mother"), and stream is treated as a fixed effect (there are few streams, and we might be interested in the effect of a particular stream due to the very large differences in temperature among them).

```
# This takes into account that mother is nested within stream
model3<-lmer(first_fl_j~stream+(1|mother_pl_id_new), REML=F,data=data_cg)
kable(prettify(summary(model3))) # Stream is significant
```

	Estimate	CI (lower)	CI (upper)	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	148.044457	146.782915	149.3357177	0.6411431	76.64303	230.907038	<0.001	***
stream: Main	3.590756	1.778078	5.3732723	0.9039719	74.07755	3.972199	<0.001	***
stream: Park	-1.520293	-3.306892	0.2427148	0.8921881	68.70894	-1.704005	0.093	.
stream: WS	6.439021	4.434251	8.4908193	1.0197833	87.65451	6.314107	<0.001	***

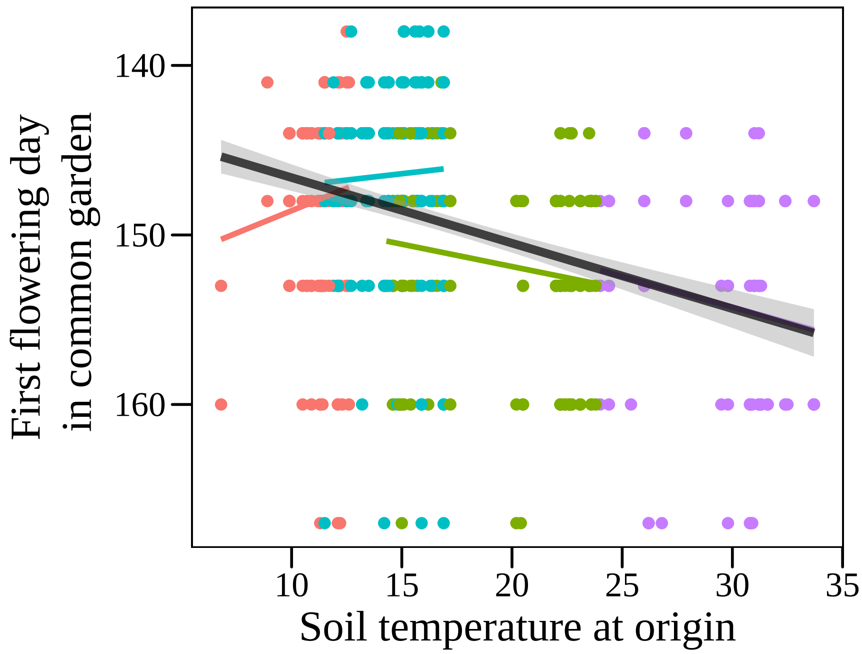
```
# Likelihood ratio test for testing the signigicance of random effects
# Compares a model with a given random effect to the same model without the random effect
rand(model3) # Mother is significant
```

```
## Analysis of Random effects Table:
##                  Chi.sq Chi.DF p.value
## mother_pl_id_new   14.9      1   1e-04 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

There is an effect of both mother plant and stream of origin on flowering phenology (first flowering day) in the common garden: there are differences among streams, and differences among mothers within streams.

Are differences among mother plants on the flowering phenology in the common garden related to soil temperature at the site of origin?

We expect plants to respond to warming in spring in a counter-gradient fashion, i.e. plants growing on warmer soils will flower early in the field but later in the common garden (requiring more warm days to start development) than plants from cold soils. Plants growing on colder soils will flower later in the field, but earlier in the common garden, because they can start developing at lower temperatures (they are adapted to colder conditions and they have evolved to compensate for the later and shorter growing season). They have the genetic capacity to develop at lower temperatures, probably because they have been selected for rapid development in environments with short growing seasons.



LM with pooled data

```
kable(prettify(summary(lm(first_fl_j~temp_ori,data=data_cg))))
```

	Estimate	CI (lower)	CI (upper)	Std. Error	t value	Pr(>|t|)	
(Intercept)	142.752022	141.2723707	144.2316742	0.7532399	189.517345	<0.001	***
temp_ori	0.386592	0.3067632	0.4664207	0.0406381	9.513048	<0.001	***

There is a strong overall relatiohsnip among FFD in the common garden and soil temperature at the origin, that goes in the expected direction (i.e. opposite to the relationship observed in field conditions).

LMM for each stream

```
kable(prettify(summary(lmer(first_fl_j~temp_ori+(1|mother_pl_id_new),data=subset(data_cg,stream=="17"))
```

	Estimate	CI (lower)	CI (upper)	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	154.3898705	141.701049	167.1512023	6.5197466	38.00169	23.6803484	<0.001	***
temp_ori	-0.5606001	-1.679326	0.5536093	0.5722251	37.53504	-0.9796847	0.334	

```
kable(prettify(summary(lmer(first_fl_j~temp_ori+(1|mother_pl_id_new),data=subset(data_cg,stream=="Main"
```

	Estimate	CI (lower)	CI (upper)	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	146.5881059	141.4157126	151.7604992	2.6398204	145.0001	55.529575	<0.001	***
temp_ori	0.2637725	-0.0012667	0.5288118	0.1352674	145.0001	1.950009	0.053	.

```r
kable(prettify(summary(lmer(first_fl_j~temp_ori+(1|mother_pl_id_new),data=subset(data_cg,stream=="Park")
```

	Estimate	CI (lower)	CI (upper)	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	150.3612790	138.751595	162.1553087	5.9804060	21.07131	25.1423197	<0.001	***
temp_ori	-0.2635643	-1.070736	0.5316711	0.4095928	21.22086	-0.6434789	0.527	

```r
kable(prettify(summary(lmer(first_fl_j~temp_ori+(1|mother_pl_id_new),data=subset(data_cg,stream=="WS"))
```

	Estimate	CI (lower)	CI (upper)	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	150.6038819	126.8138383	175.621368	12.3552238	12.27455	12.1894904	<0.001	***
temp_ori	0.1623841	-0.6869118	0.973925	0.4219175	12.08425	0.3848718	0.707	

Within each stream, there are no significant relationships.

LMM including temperature, stream and mother

```r
# This takes into account that mother is nested within stream
model4<-lmer(first_fl_j~temp_ori+(1|stream)+(1|mother_pl_id_new),data = data_cg)
kable(prettify(summary(model4))) # Temperature is significant
```

	Estimate	CI (lower)	CI (upper)	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	144.461930	140.5537963	148.6148916	2.0209199	5.814641	71.48325	<0.001	***
temp_ori	0.306965	0.1107079	0.5046474	0.0975703	9.032306	3.14609	0.012	*

```r
rand(model4) #Stream and mother are significant
```

```
## Analysis of Random effects Table:
##                  Chi.sq Chi.DF p.value
## stream              8.5      1   0.004 **
## mother_pl_id_new   13.8      1   2e-04 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Differences among mother plants in phenology are related to soil temperature at origin. Plants respond to warming a counter-gradient fashion: plants from warmer soils flower later in the common garden than plants from cold soils.