Analysis feeding rate and cardenolides sequestration for 4 species of aphids on 20 genotypes of milkweed A.syriaca

Method

Digested data from Zust and Agrawal's study is used throughout this analysis [1].

Different aphid-plant combinations are observed for aphid growth and descriptive characters. There are 4 species of aphid included. Their diets range from monophagous specialist to wide generalist [2], with M.per being a broad generalist, A.asc a narrow generalist, A.ner being a specialist and M.asc a monophagous specialist.. Twenty genotypes of milkweed *A.syriaca* are included as host plants to allow comparison of species performances [2].

Feeding rate:

Zust and Agrawal estimated the feeding rate of aphids on different genotypes of *A. syriaca.* [2] The honeydew is exuded by the aphid as waste product and reflects the phloem consumption and respiratory rate. [3] To quantify the food consumption, per capita honeydew is calculated and log-transformed to meet the homoscedasticity assumption.

An ANOVA is run with transformed feeding rate as response variable and aphid species as explanatory variable to assess feeding rates across species.

To evaluate the effect of host genotypes, another ANOVA was done but with plant genotype as the only explanatory variable. A further 2-way ANOVA was done with interaction term included.

Cardenolides Sequestration:

Previous studies showed that Cardenolides is produced by *A. syriaca* as an important form of plant defence against insect herbivores [4] and the degree of diet specialization correlates with sequestration of plant toxin [5]. Zust and Agrawal thus hypothesised that more specialised aphid would tolerate a higher level of in-body cardenolide [2].

From an ANOVA of log(c11:5) against species and sample weight, it was found that sample weight partially mask the effect of species. To unmask the effect of interest, ANOVA is carried out of the sample weight on genotype, species and their interaction (see Table 1). After discarding the insignificant term of genotype, we regressed the sample weight on the remaining terms and took the residuals as the independent variation of sample weight.(termed SW_effect4). (Line 330)

Table 1 ANOVA of sample weight for chemicals, which is found to correlate with factors of interest.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	3	1393.08	464.36	97.33	0.0000
Species:Genotype	76	879.45	11.57	2.43	0.0001
Residuals	75	357.82	4.77		

ANOVA is carried out for with log-transformed concentration of cardenolide as response variable and species as explanatory variable to assess inter-species difference. A second ANOVA with genotype solely as explanatory variable. And a third including the interaction between the two. This set of analysis is done for 3 most prevalent in-body cardenolides [2], including c11:5, c12:2 and c12.8.

Results

Feeding rate

ANOVA shows that different species of aphids vary significantly in their feeding rate. (F=66.26, P=4.84E(-33),R-squared=39.38). *M. asc* and *A. ner* has the highest mean feeding rates, stastically indifferent from each other (see Figure 1). Mean feeding rate of *A. ner* and *M. asc* is statistically indifferent (t=2.059, DOF=306,P=0.040). *M. per* has the lowest mean feeding rate, 1.94 unit lower than that of *M. asc*.

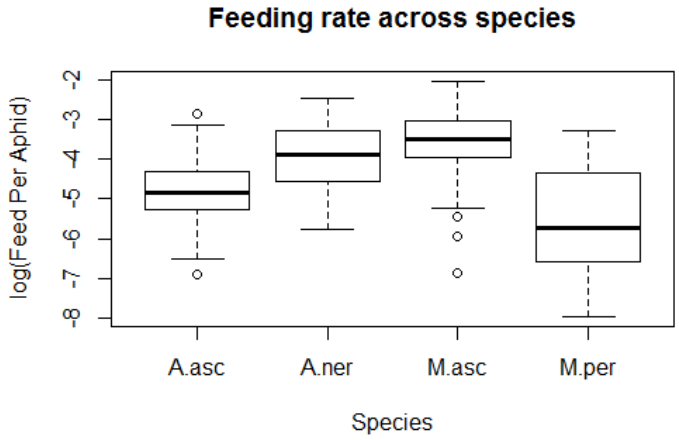

Figure 1 Feeding rate vary significantly across species

Across species, feeding rates for aphids grown on different genotypes of host plants, however, don't differ significantly (F=0.616, P=0.894).

Including the interaction term, ANOVA shows that species effect on feeding rate is genotype-dependent (F=1.439, P=0.0212). Genotype effect on feeding rate is then assessed within each species. Only for *M. asc*, feeding rate is affected by genotype (F=1.815 df = (19; 57), p<0.05) while no significant effect in other species (see Table 2). Interestingly, *M. per* shows an unusual high residual standard error of 1.205 while others have their RSE around 0.75.

Table 2 Summary of Species-Specific linear regression of feeding rate against genotype.[]

Independent Variable: Genotype		*Dependent variable:* Feed Per Aphid		
	Species			
	A. asc	*A. ner*	*M. asc*	*M. per*
Observations	76	78	77	79

R^2	0.288	0.362	0.377	0.297
Adjusted R^2	0.046	0.153	0.169	0.070
Residual Std. Error	0.800 (df = 56)	0.729 (df = 58)	0.767 (df = 57)	1.205 (df = 59)
F Statistic	1.191 (df = 19; 56)	1.730* (df = 19; 58)	1.815** (df = 19; 57)	1.310 (df = 19; 59)
				*p<0.1; **p<0.05; ***p<0.01

The generalist *M. per* exhibits the greatest range with a biggest error and the narrow generalist shows a less error. In contrast, specialist *M. asc* shows a higher but narrower range with a smaller error, with *A. ner* following a shifted track.

Cardenolide sequestration

M.asc sequester the highest. A.asc and A.ner sequester similar amount. M.per sequester the lowest. (see Figure 2)

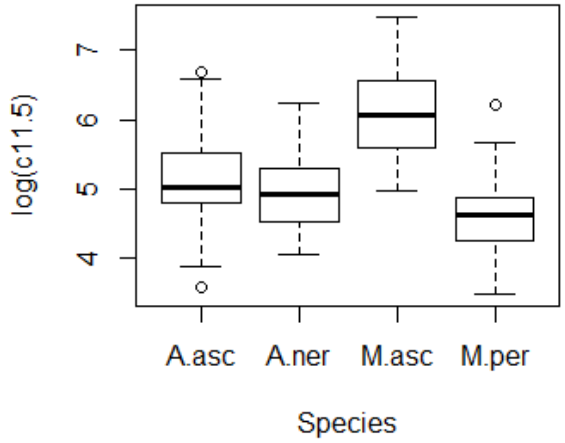

Figure 2 Species-specific c11:5 level

Of the three in-body cardenolide (c11:5, c12:2, c12:8) levels examined, all vary significantly for different species and for different sample-weight (detangled from collinearity) , while none is affected by the plant genotype. The species effect for c11:5 , however, depends on the genotype , while variation by species in c12:2 and c12:8 is statistically unaffected by genotype (See Table 3).

Table 1 ANOVA of c11.5, c12.2 and c12.8

c11.5	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**

Species	3	40.91	13.64	56.20	0.0000
SW.effect4	1	3.48	3.48	14.35	0.0003
Species:Genotype	76	30.21	0.40	1.64	0.0184
Residuals	71	17.23	0.24		
C12.2	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Species	3	45.07	15.02	59.73	0.0000
SW.effect4	1	6.44	6.44	25.62	0.0000
Species:Genotype	76	25.77	0.34	1.35	0.0994
Residuals	74	18.61	0.25		
c12.8	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species	3	32.77	10.92	76.61	0.0000
SW.effect4	1	3.19	3.19	22.35	0.0000
Species:Genotype	76	15.21	0.20	1.40	0.0726
Residuals	74	10.55	0.14		

Species-specific ANOVAs reveal that, in *M. per* subset, genotype explains variation significantly in c11:5 level (F=3.70, df=(19:18), P=0.0038) while poorly in c12:8 level (F=1.56, P=0.175). Detangled subset sample weight (termed SW.effect.Speices-name) explains c11:5 variation in both *A. asc* and *M. asc* subset (F=6.81, P=0.018 ; F=5.10 P=0.037) and c12:8 in *A. asc* subset (F=4.89, P=0.041). For *A. ner* subset, no explanatory variable is significant for any cardenolide level (F<1).

Discussion

Feeding rate

The feeding rate increases as the diet specialization increases, indicate that the specialist aphid is consuming more phloem since they are able to exudate plant toxin, although it could be the specialist is using resource less efficiently. The fact that M.asc is highly sensitive to the plant genotype confirms its high degree of diet specialization. Feeding rate of M.per and of A.asc varies randomly across the genotypes, corresponding to their highly generalised diets where difference between plants scarcely affect its activity. However M.per shows a random feeding rate even on entities of a same genotype (average SE=1.205), possibly responding vigorously to some factor that is not controlled in the experiment.

In-body Cardenolides levels

All three cardenolides here are apolar, they exhibit an overall similar variation responding to species and genotype, while responding differently to species-specific effect of genotype. M.asc sequester the highest amount, corresponding to a high consumption of phloem. The

rest 3 species exhibit a similar amount of cardenolides, indicating the process of cardenolides in M.asc is radically different.

In M.per, the broad generalist, the level of c11:5 is significantly correlates with plant genotype. A residual square mean of 2.47 compared to an average of 5 for the other 3 species. Contrast to its random distribution of feeding rate, c11:5 is consistent for the same genotype, suggesting the sequestration is independent of feeding rate.

Though not of interest, the weight of dried aphid has a significant negative effect on the cardenolide level (for c11:2, slope=-0.0994+-0.0265), which could indicate that a larger population sequester less cardenolides, or bigger aphids are more efficient at excretion. In lack of relevant information we are unable to perform tests.

References:

[1] Honeydew dataset,https://moodle.ucl.ac.uk/mod/resource/view.php?id=2139811 and chemicals dataset, https://moodle.ucl.ac.uk/mod/resource/view.php?id=2139815

[2] Züst T, Agrawal AA (2015) Population growth and sequestration of plant toxins along a gradient of specialization in four aphid species on the common milkweed Asclepias syriaca. Functional Ecology, online in advance of print. http://dx.doi.org/10.1111/1365-2435.12523

[3] Dixon, A.F.G. (1998) Aphid Ecology, 2nd edn. Chapman & Hall, London.

[4] Agrawal, A.A., Petschenka, G., Bingham, R.A., Weber, M.G. & Rasmann, S. (2012) Toxic cardenolides: chemical ecology and coevolution of specialized plant-herbivore interactions. New Phytologist, 194, 28–45.

[5] Lampert, E.C., Dyer, L.A. & Bowers, M.D. (2014) Dietary specialization and the effects of plant species on potential multitrophic interactions of three species of nymphaline caterpillars. Entomologia Experimentalis Et Applicata, 153, 207–216.

Appendix

```
setwd("C:/Users/Feng/Google Drive/Of interests/BIOL7015_RData/CW_Aphid")
dir()

#Load and process the data.
honeydew<-read.delim('Honeydew.txt')
chemicals<-read.delim('Chemicals.txt')
chemicals$Genotype<-as.factor(chemicals$Genotype)
honeydew$Genotype<-as.factor(honeydew$Genotype)
chemicals$logc11.5<-log(chemicals$c11.5)
chemicals$logc12.2<-log(chemicals$c12.2)
chemicals$logc12.8<-log(chemicals$c12.8)
honeydew$FeedPerAphid=log(honeydew$Honeydew.mass/honeydew$Cumulative.aphids)


#Detangle Sample Weight from factors of interest

##The effect of Sample.weight is dependent on the Species
lm.logc11.5_S_Sam<-lm(logc11.5~SW.effect+Species,data=chemicals)
hist(lm.logc11.5_S_Sam$res)
summary(lm.logc11.5_S_Sam)
```

```
summary(aov(Sample.weight~Species,data=chemicals))
#Significant linearity between Sample.weight and Species
summary(aov(Sample.weight~Genotype,data=chemicals))
#insignificant association with genotype alone
summary(SW.effect4.aov<-aov(Sample.weight~Species+Species:Genotype,data=chemicals))
#genotype-species interaction is significant
summary(SW.effect4.aov<-aov(Sample.weight~Species+Species:Genotype,data=chemicals))
SW.effect4<-residuals(lm(Sample.weight~Species+Species:Genotype,data=chemicals))

#Assess the effect of detangled samplew weight alone
aov.logc11.5<-aov(logc11.5~SW.effect4,data=chemicals)
summary(aov.logc11.5)
summary(lm(logc11.5~SW.effect4,data=chemicals))

##Analysis of feeding rates
#analyse feeding rates across species
aov.fdrate<-aov(FeedPerAphid~Species,data=honeydew)
summary(aov.fdrate)
lm.fdrate<-lm(FeedPerAphid~Species,data=honeydew)
plot(FeedPerAphid~Species,data=honeydew,main='Feeding rate across species',xlab='Aphid
Species',ylab='log(Feed Per Aphid)')
#Figure 1

#Across genotypes
aov.fdrate.geno<-aov(FeedPerAphid~as.factor(Genotype),data=honeydew)
summary(aov.fdrate.geno)
lm.fdrate.geno<-lm(FeedPerAphid~as.factor(Genotype),data=honeydew)
summary(lm.fdrate.geno)

#Aov including host-genotype and species
aov.fdrate0<-aov(FeedPerAphid~Species*as.factor(Genotype),data=honeydew)
summary(aov.fdrate0)
lm.fdrate1<-lm(FeedPerAphid~as.factor(Genotype):Species+Species,data=honeydew)
summary(lm.fdrate1)

#Custom a function to do species specifci anova
(func3<-function(spec,lm.p=F){
 spec<-as.character(spec)
 honeydew<-na.omit(honeydew)
 data=honeydew[honeydew$Species==spec,]
 print(summary(aov<-aov(FeedPerAphid~Genotype,data=data)))
 (lm<-lm(FeedPerAphid~Genotype,data=data))
 if(lm.p) {print(summary(lm))}
 return(lm)
})
lapply(levels(honeydew$Species),func3)->lm.geno.InSp

#Produce Table 2
stargazer(lm.geno.InSp[[1]],
        lm.geno.InSp[[2]],
        lm.geno.InSp[[3]],
```

```
        lm.geno.InSp[[4]],
        model.names = T,
        multicolumn = T,
        out = 'inSp.html' ,
        type='html'
)

#Analysis of c11:5 level
#Produce Figure 2
plot(y=c(logc11.5),x=Species,data=chemicals,xlab='Species',ylab='log(c11.5)')

#a)Assess the effect of Species on c115
aov.logc11.5_Sp<-aov(logc11.5~Species+SW.effect4,data=chemicals) #SW.effect4 absorbs
variation due to SW.
summary(aov.logc11.5_Sp)
## Different species of aphids differ in their c115 level signifcantly.

#b)Assess the effect of Genotype on c115
aov.logc11.5_Geno<-aov(logc11.5~Genotype+SW.effect4,data=chemicals)
summary(aov.logc11.5_Geno)
##From this ANOVA,across species, aphids raised on different genotypes don't differ in the
c115 level

#c)Assess interaction between species and genotype
aov.logc11.5_SpbyGeno<-
aov(logc11.5~Species+Species:Genotype+SW.effect4,data=chemicals)
summary(aov(logc11.5~Species+Species:Genotype+SW.effect4,data=chemicals))
## The effect of Species differs for different genotype, though weakly (F=1.638,P=0.0184)

#Species-specific analysis
#A.asc, same for other species upon replacement
Sub_A.asc<-subset(chemicals,Species=='A.asc')
SW.effect.A.asc<-residuals(lm(Sample.weight~Genotype,data=Sub_A.asc))
aov.logc11.5_Geno.A.asc<-aov(logc11.5~Genotype,data=Sub_A.asc)
summary(aov.logc11.5_Geno.A.asc)
aov.logc11.5_GenobySW.A.asc<-aov(logc11.5~Genotype+SW.effect.A.asc,data=Sub_A.asc)
summary(aov.logc11.5_GenobySW.A.asc)
lm.logc115.5_GenobySW.Mper<-
lm(logc11.5~Genotype+SW.effect4,data=chemicals,subset=which(Species=='M.per'))
summary(lm.logc115.5_GenobySW.Mper)

#Custom function for repeating export of tables
(Ptable<-function(aov,file='default')
{
  library(R2HTML)
  library(xtable)
  print(xtable(aov),type='html')->tmp
  name<-aov

  target <- HTMLInitFile(getwd(),filename=as.character(file), BackGroundColor="#BBBBEE")
  HTML(tmp,file=target)
```

```
}
)
#Produce table 3
Ptable(aov.logc11.5_SpbyGeno)
Ptable(aov.logc12.2_SpbyGeno)
Ptable(aov.logc12.8_SpbyGeno)
```