Integrating quantitative and molecular genetics in host-range determination

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Mission of Foreign Disease - Weed Science Research Unit

- Find and develop foreign plant pathogens for classical biological control of introduced invasive weeds in the U.S.

Steps in Developing Plant Pathogens for Classical Biological Control of Weeds

- Find diseases of introduced weeds in the native (foreign) ranges of the weeds
- Isolate the pathogens
- Test the pathogens for damage
- Test the pathogens for their host range (safety)

Target:

- Russian thistle (*Salsola tragus*)

Pathogens:

- *Colletotrichum gloeosporioides* f. sp. *salsolae* (CGS)
  - Facultative parasitic fungus
  - Hungary (other isolates from Greece and Russia)
- *Uromyces salsolae*
  - Obligate parasitic rust fungus
  - Russia

Tumbleweed/Russian Thistle

Colletotrichum gloeosporioides f. sp. *salsolae* (CGS)

Uromyces salsolae

Problem

How to best evaluate disease reaction of non-target plant species?

- Avoid discarding potentially beneficial organisms
- Ensure pathogen safety

Partial Disease Severity Results (CGS)

Table. Plants in the Chenopodiaceae

Genus	Species	No. Plants		Average Disease Rating
		Inoc	Pos	
Grayia	spinosa	10	0	0
Kochia	scoparia	15	0	0
Nitrophila	occidentalis	7	7	1.8
Salicornia	bigelovii	1	1	4.0
Salicornia	maritima	5	5	3.6
Salicornia	virginica	5	0	0
Salsola	kali	11	9	2.4
Salsola	orientalis	13	2	0.4
Salsola	paulsenii	5	5	4.0
Salsola	tragus	261	252	2.9
Salsola	australis	65	55	1.1
Suaeda	californica	1	0	0
Suaeda	taxifolia	4	4	2.2

NEED

- Environmentally-independent measure of disease severity
- Disease severity for <u>species</u> (vs. individual plants)
- An objective indicator of susceptible & non-susceptible <u>species</u>
- A means to evaluate rare and difficult or impossible to grow species
- A means to objectively compare disease on target vs. non-targets

Partial Solution
(next best)

- **Use ranks of disease severity ratings**
- **Use logit values of disease incidence**
- **Analyze using weighted mixed model ANOVA**
- **Produce least squares mean estimates**

Full Solution

Combine disease evaluation data with another statistical approach

- Incorporate all available knowledge
- Incorporate genetic relationships among species with variances and disease evaluation data
- Get broad-based predictions for species

Mixed Model Equations (MME) and Best Linear Unbiased Predictors (BLUPs)

- **Long-established quantitative genetics approach**

- **Standard in generation of breeding values (BLUPs) for dairy cattle worldwide.**
  - **C. R. Henderson – 30+ years of publications from 1949**

- **Also for quarter horses, swine, trees, crop plants,…………….**

- **Common objective:**
  - **Predict breeding values (genetic merit of potential parents) -BLUPs**

BLUPs

Best – minimum mean squared error

Linear – linear function of the data

Unbiased – average value of the estimate equals average value of quantity being estimated; no estimable function bias

Predictor – realized value of a random variable

BLUPs are also parametric Bayes estimates

Value of the MME & BLUPs in Host Range Determination

- Predict susceptibility of plant <u>species</u> relative to that of the target species
- Use all available information
  - disease ratings or incidence
  - variance/covariance structure
  - genetic relationships among species
- Predict disease reactions for species that cannot be tested
- Determine the complete host-range of a pathogen among both tested and not tested species
- Determine relevant lists of non-target species to test

How?

- Incorporate genetic relationships
- Incorporate genetic variances
- Incorporate performance (disease evaluation) data

How?

Incorporate genetic relationships from:

- Pedigrees and coefficients of co-ancestry

OR

- DNA sequences and genetic distance matrices

How?

Incorporate genetic variances from

- Heritability estimates

OR

- Estimate variance from data

How?

Incorporate performance data (disease/damage evaluation data)

How?

- Integrate data into Mixed Model Equations
- Generate Best Linear Unbiased Predictors (BLUPs) for species

How?

- Generate DNA sequences, ITS (and/or other), for plant species
- Generate distance matrix among species based on these sequences
- Integrate these genetic distances with performance data
- Run the MME to generate BLUPs

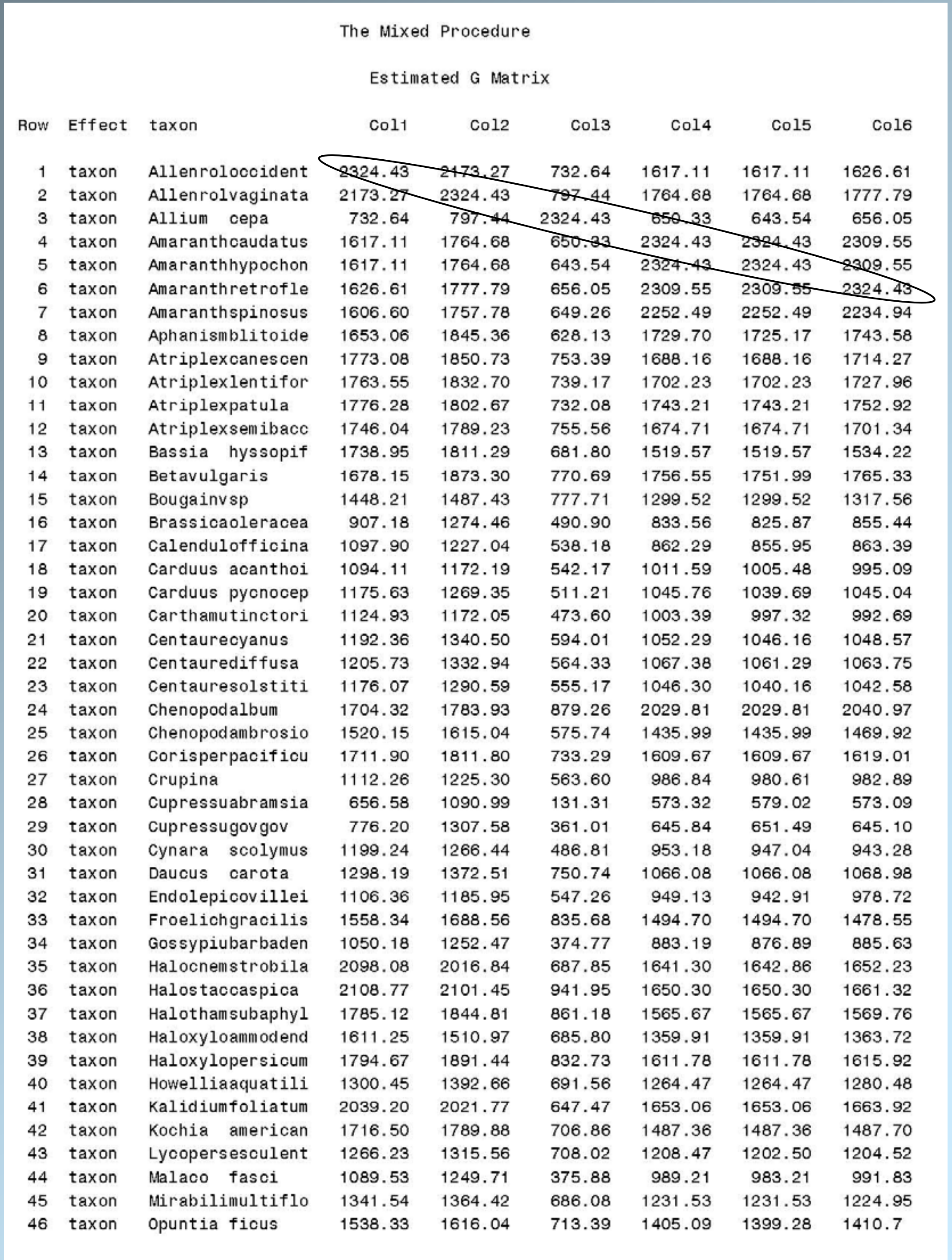
Model
(in matrix notation)

- $y = XW + ZU + E$
  - y = n × 1 vector of ranks of disease ratings
  - X = n × 1 design vector of "1"s for the fixed intercept only
  - W = 1 × 1 unknown vector for the fixed effect parameters, in this case only the intercept
  - Z = n × j design matrix for the random effects, in this case species
  - U = j × 1 unknown vector of the random effects parameters
  - E = n × 1 vector of residuals (errors)
- variance of $y = S = ZGZ' + R$
  - $G = j \times j$ matrix of variances and covariances from distance matrix of DNA sequences and variance among species
  - $R = n \times n$ error matrix with known error variance as estimated from data
- $\hat{U} = GZ'\hat{S}^{-1}(y - X\hat{W})$ = BLUPs

Partial G matrix

relationship matrix=(1-each element of distance matrix)

G= Variance among species × each element of relationship matrix



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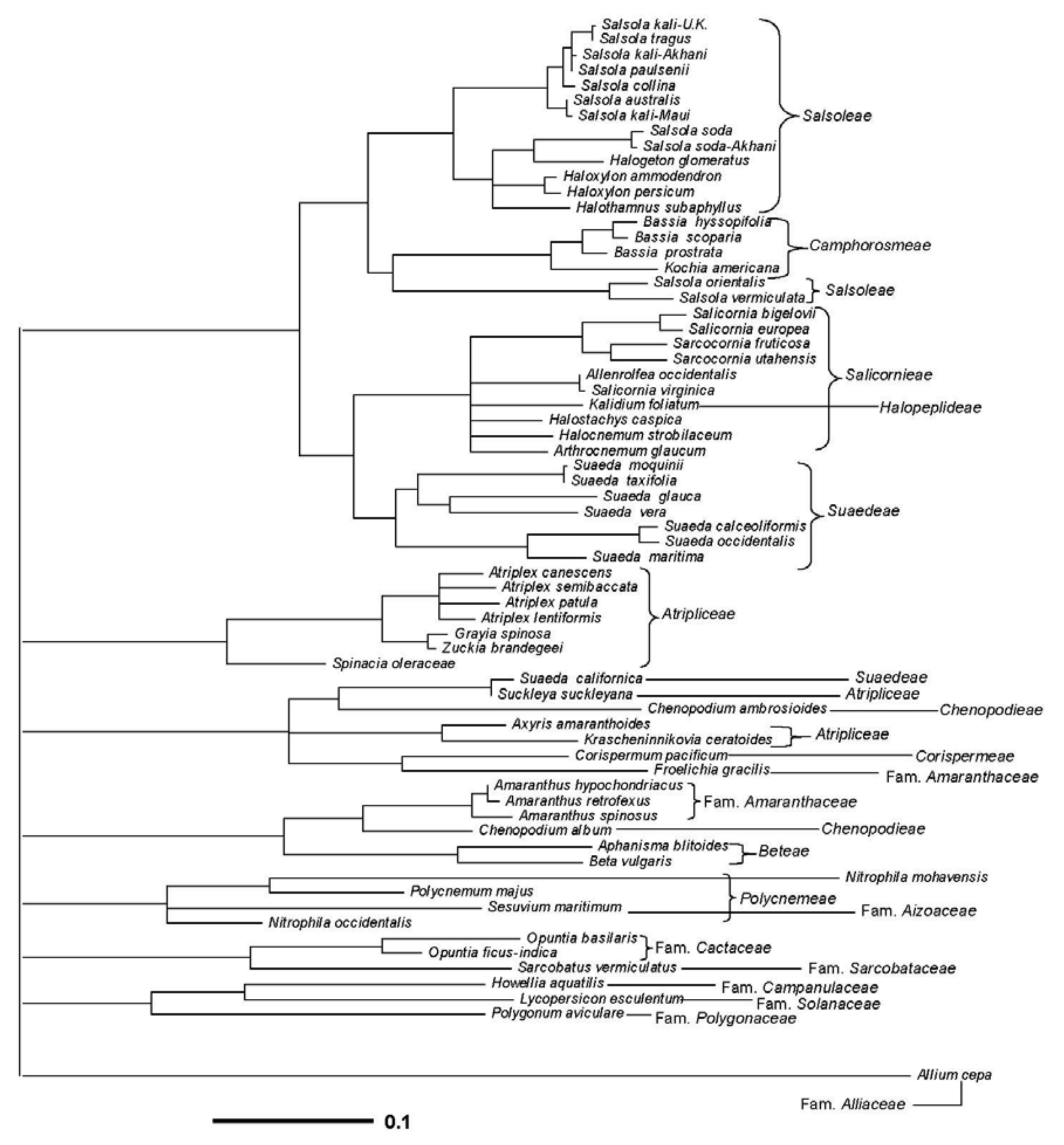
                              The Mixed Procedure

                               Estimated G Matrix

Row  Effect  taxon               Col1       Col2       Col3       Col4       Col5       Col6

  1  taxon   Allenroloccident  2324.43    2173.27     732.64    1617.11    1617.11    1626.61
  2  taxon   Allenrolvaginata  2173.27    2324.43     797.44    1764.68    1764.68    1777.79
  3  taxon   Allium  cepa       732.64     797.44    2324.43     650.33     643.54     656.05
  4  taxon   Amaranthcaudatus  1617.11    1764.68     650.33    2324.43    2324.43    2309.55
  5  taxon   Amaranthhypochon  1617.11    1764.68     643.54    2324.43    2324.43    2309.55
  6  taxon   Amaranthretrofle  1626.61    1777.79     656.05    2309.55    2309.55    2324.43
  7  taxon   Amaranthspinosus  1606.60    1757.78     649.26    2252.49    2252.49    2234.94
  8  taxon   Aphanismblitoide  1653.06    1845.36     628.13    1729.70    1725.17    1743.58
  9  taxon   Atriplexcanescen  1773.08    1850.73     753.39    1688.16    1688.16    1714.27
 10  taxon   Atriplexlentifor  1763.55    1832.70     739.17    1702.23    1702.23    1727.96
 11  taxon   Atriplexpatula    1776.28    1802.67     732.08    1743.21    1743.21    1752.92
 12  taxon   Atriplexsemibacc  1746.04    1789.23     755.56    1674.71    1674.71    1701.34
 13  taxon   Bassia  hyssopif  1738.95    1811.29     681.80    1519.57    1519.57    1534.22
 14  taxon   Betavulgaris      1678.15    1873.30     770.69    1756.55    1751.99    1765.33
 15  taxon   Bougainvsp        1448.21    1487.43     777.71    1299.52    1299.52    1317.56
 16  taxon   Brassicaoleracea   907.18    1274.46     490.90     833.56     825.87     855.44
 17  taxon   Calendulofficina  1097.90    1227.04     538.18     862.29     855.95     863.39
 18  taxon   Carduus acanthoi  1094.11    1172.19     542.17    1011.59    1005.48     995.09
 19  taxon   Carduus pycnocep  1175.63    1269.35     511.21    1045.76    1039.69    1045.04
 20  taxon   Carthamutinctori  1124.93    1172.05     473.60    1003.39     997.32     992.69
 21  taxon   Centaurecyanus    1192.36    1340.50     594.01    1052.29    1046.16    1048.57
 22  taxon   Centaurediffusa   1205.73    1332.94     564.33    1067.38    1061.29    1063.75
 23  taxon   Centauresolstiti  1176.07    1290.59     555.17    1046.30    1040.16    1042.58
 24  taxon   Chenopodalbum     1704.32    1783.93     879.26    2029.81    2029.81    2040.97
 25  taxon   Chenopodambrosio  1520.15    1615.04     575.74    1435.99    1435.99    1469.92
 26  taxon   Corisperpacificu  1711.90    1811.80     733.29    1609.67    1609.67    1619.01
 27  taxon   Crupina           1112.26    1225.30     563.60     986.84     980.61     982.89
 28  taxon   Cupressuabramsia   656.58    1090.99     131.31     573.32     579.02     573.09
 29  taxon   Cupressugovgov     776.20    1307.58     361.01     645.84     651.49     645.10
 30  taxon   Cynara  scolymus  1199.24    1266.44     486.81     953.18     947.04     943.28
 31  taxon   Daucus  carota    1298.19    1372.51     750.74    1066.08    1066.08    1068.98
 32  taxon   Endolepicovillei  1106.36    1185.95     547.26     949.13     942.91     978.72
 33  taxon   Froelichgracilis  1558.34    1688.56     835.68    1494.70    1494.70    1478.55
 34  taxon   Gossypiubarbaden  1050.18    1252.47     374.77     883.19     876.89     885.63
 35  taxon   Halocnemstrobila  2098.08    2016.84     687.85    1641.30    1642.86    1652.23
 36  taxon   Halostaccaspica   2108.77    2101.45     941.95    1650.30    1650.30    1661.32
 37  taxon   Halothamsubaphyl  1785.12    1844.81     861.18    1565.67    1565.67    1569.76
 38  taxon   Haloxyloammodend  1611.25    1510.97     685.80    1359.91    1359.91    1363.72
 39  taxon   Haloxylopersicum  1794.67    1891.44     832.73    1611.78    1611.78    1615.92
 40  taxon   Howelliaaquatili  1300.45    1392.66     691.56    1264.47    1264.47    1280.48
 41  taxon   Kalidiumfoliatum  2039.20    2021.77     647.47    1653.06    1653.06    1663.92
 42  taxon   Kochia  american  1716.50    1789.88     706.86    1487.36    1487.36    1487.70
 43  taxon   Lycopersesculent  1266.23    1315.56     708.02    1208.47    1202.50    1204.52
 44  taxon   Malaco  fasci     1089.53    1249.71     375.88     989.21     983.21     991.83
 45  taxon   Mirabilimultiflo  1341.54    1364.42     686.08    1231.53    1231.53    1224.95
 46  taxon   Opuntia ficus     1538.33    1616.04     713.39    1405.09    1399.28    1410.7
```

Phylogram from ITS sequence data



Power

- Beta (β) = probability of committing a Type II error and not rejecting a false null hypothesis
- Declaring no significant difference from zero when a difference exists
- Declaring a species not susceptible when it probably is
- Power = 1-β, probability of correctly rejecting a false null hypothesis
- Power values $\geq$ 0.80 are generally regarded as significant

Least squares means

59 species evaluated

6 susceptible (non-zero) species

CV= 3.31 to 407.87%

	CGS				***Uromyces salsolae***			
Genus species	**Least squares means estimates**	**Standard error of estimate**	**Pr>|t|**	**Power (1-β)**	**Least squares means estimates**	**Standard error of estimate**	**Pr>|t|**	**Power (1-β)**
***Salsola kali*-U.K.**	**285.40**	**41.47**	**<0.0001**	**0.167**	**132.10**	**10.89**	**<0.0001**	**0.082**
Salsola tragus	**277.82**	**37.46**	**<0.0001**	**0.202**	**141.59**	**3.71**	**<0.0001**	**0.132**
Salsola collina	**285.98**	**35.00**	**<0.0001**	**0.235**	**62.05**	**0.54**	**NS**	**0.781**
Salsola paulsenii	**296.40**	**34.19**	**<0.0001**	**0.248**	**120.34**	**44.09**	**NS**	**<0.050**
***Salsola kali*-Akhani**	**NT**	**NT**	**---------**	**------**	**NT**	**NT**	**---------**	**------**
Salicornia bigelovii	**247.06**	**17.30**	**<0.0001**	**0.999**	**61.21**	**2.22**	**NS**	**0.321**
Salsola australis	**111.24**	**12.72**	**NS**	**0.996**	**60.20**	**3.38**	**NS**	**0.200**
***Salsola kali*-Maui**	**111.08**	**18.00**	**NS**	**0.884**	**36.67**	**52.20**	**NS**	**<0.050**
Salicornia europaea	**144.18**	**16.09**	**NS**	**0.922**	**60.03**	**4.76**	**NS**	**0.148**
Sarcocornia fruticosa	**NT**	**NT**	**---------**	**------**	**NT**	**NT**	**---------**	**------**
Bassia hyssopifolia	**239.08**	**29.50**	**<0.0001**	**0.355**	**52.64**	**39.61**	**NS**	**<0.050**
Bassia scoparia	**110.60**	**10.35**	**NS**	**>0.999**	**NT**	**NT**	**---------**	**------**
Nitrophila occidentalis	**254.37**	**81.79**	**NS**	**<0.100**	**60.20**	**3.38**	**NS**	**0.200**
Halothamnus subaphyllus	**NT**	**NT**	**---------**	**------**	**NT**	**NT**	**---------**	**------**

46 species evaluated

3 susceptible (non-zero) species

CV= 2.6 to 142.4%

BLUPs

89 species evaluated

30 susceptible (non-zero) species

CV= 3.53 to 43.32%

	CGS				*Uromyces salsolae*			
Genus species	BLUP	Standard error of prediction	Pr>|t|	Power (1-β)	BLUP	Standard error of prediction	Pr>|t|	Power (1-β)
Salsola kali-U.K.	247.92	10.23	<0.0001	>0.999	102.94	5.30	0.006	>0.999
Salsola tragus	246.73	9.84	<0.0001	>0.999	101.97	5.10	0.007	>0.999
Salsola collina	235.40	10.70	<0.0001	>0.999	95.61	5.33	0.019	0.995
Salsola paulsenii	225.51	7.59	<0.0001	>0.999	96.90	4.65	0.023	>0.999
Salsola kali-Akhani	224.75	7.93	<0.0001	>0.999	97.02	4.85	0.023	>0.999
Salicornia bigelovii	213.39	14.95	<0.0001	>0.999	54.47	7.93	NS	>0.999
Salsola australis	208.14	10.29	<0.0001	>0.999	95.47	4.94	0.028	>0.999
Salsola kali-Maui	207.53	10.58	0.0001	>0.999	92.71	6.23	0.037	>0.999
Salicornia europaea	205.02	14.86	0.0002	0.996	54.55	7.93	NS	>0.999
Sarcocornia fruticosa	190.04	21.67	0.0027	0.978	55.43	9.21	NS	0.959
Bassia hyssopifolia	188.26	17.62	0.0026	0.960	67.32	9.44	NS	0.983
Bassia scoparia	187.80	9.01	0.0036	>0.999	68.09	9.72	NS	0.923
Nitrophila occidentalis	184.10	16.80	0.0070	>0.999	-------	-------	------	------
Halothamnus subaphyllus	176.36	18.05	0.0152	>0.999	82.58	8.10	NS	0.995

66 species evaluated

7 susceptible (non-zero) species; all *Salsola* spp.

CV= 4.99 to 36.74%

BLUPs and the binary/binomial case

- Data converted to “1” or “0”

- Disease vs no disease

- Successes/attempts = x, e.g., 0.10, 0.20, 0.90, etc.

- Proportions of 0 and 1 set to 0.01 and 0.99, respectively.

- Logit transformation: log(x/1-x) or log(odds)

Odds ratio

- Odds ratio= the natural logarithm to the power of the BLUP for each species, i.e., e^{BLUP} for logit values

- A probability of whether disease is likely

- Odds ratios > 1 indicate disease occurrence is likely

- The larger the odds ratio the greater the likelihood of disease

BLUPs

89 species evaluated

26 species with significant disease incidence

15 species with odds ratios greater than 1
-5 native spp.

Genus species[1]	CGS				*Uromyces salsolae*			
	BLUP (Logit)	Standard error of prediction	Pr>|t|	Odds ratio	BLUP (Logit)	Standard error of prediction	Pr>|t|	Odds ratio
Salsola kali- U.K.	12.11	2.51	<0.0001	79.12	8.49	2.51	0.002	9.19
Salsola tragus	12.00	2.51	<0.0001	70.67	8.31	2.51	0.002	7.49
Salsola collina	10.94	2.55	<0.0001	24.48	7.12	2.52	0.007	1.62
Salsola paulsenii	9.94	2.77	0.0005	8.98	7.22	2.70	0.011	2.71
Salsola kali- Akhani	9.85	2.77	0.0006	8.24	7.24	2.70	0.011	2.78
Salicornia bigelovii	10.53	2.68	0.0002	16.31	0.06	2.69	NS	0.01
Salsola australis	8.76	2.55	0.0009	2.78	6.82	2.71	0.016	1.64
Salsola kali- Maui	8.81	2.57	0.0009	2.92	6.46	2.61	0.017	0.78
Salicornia europaea	9.46	2.64	0.0005	5.56	-0.01	2.69	NS	0.01
Sarcocornia fruticosa	7.93	2.92	0.0078	1.21	0.39	2.80	NS	0.02
Bassia hyssopifolia	9.27	2.86	0.0016	4.61	2.72	2.81	NS	0.08
Bassia scoparia	8.99	2.95	0.0030	3.50	2.54	2.88	NS	0.07
Nitrophila occidentalis	8.09	3.08	0.0101	1.42	-------	-------	------	------
Halothamnus subaphyllus	6.80	3.18	0.0351	0.39	4.62	3.05	NS	0.39

66 species evaluated

8 species with significant disease incidence

7 species with odds ratios greater than 1
-no native spp.

Why?

- The large number of inter-specific relationships place the disease reaction of each species in context, genetically and mathematically, with **all** species analyzed.

- A spider-web analogy is apt: in the case of CGS there are 89 × 89 = 7921 interconnected nodes in the G matrix, and a force, e.g., disease, applied to any node “pulls” the other nodes, and web, in that direction. Forces at other nodes “pull” in opposing directions.

- Or, in the case of CGS, BLUPs reflect 59 × 59 (species with data) ÷ 2 = 1,740 fractional replications based on the genetic inter-relationships among these species.

- BLUPs reflect the disease reactions of each species plus the disease reactions of **all** of the other inter-related species.

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- **BLUPs are environmentally independent**

Advantages continued

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- **BLUPs predict <u>species</u> performance (vs. averages from the material tested in a greenhouse)**
- **The mixed model equations and BLUPs can be used to construct test plant lists**

Further Implications

- Useable with any agent and any target
- Useable in *ex-post* analyses
- Useable with historical data
- Useable by anyone

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- Why doesn't there seem to be a standard objective criterion for evaluation of susceptibility/damage?

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- Why is the average of a few leaf spots/chews/eggs (or lack thereof) on an infinitesimal sample of a species deemed representative of the species as a whole?

- Why doesn't there seem to be a standard objective criterion for evaluation of susceptibility/damage?

- Why isn't probability of susceptibility/damage that criterion?

Questions

- Is this species susceptible based on one pustule on one leaf of one plant in a greenhouse test?

Uromyces salsolae

- Probably not: BLUP=63.3; Pr>|t|=0.95; odds ratio=0.013
- Doubtful that even the plant is susceptible
- Initial APHIS feedback seems to disagree

Questions

•Whatever happened to the scientific method and rigorous hypothesis testing?

$$Ho: \mu_1 = \mu_2 = \mu_n = 0$$

•Is this not necessary in biological control of weeds?

•Or is science simply not necessary for regulators?

Recent Publications

- Berner, D.K. 2010. BLUP, a new paradigm in host-range determination. Biological Control 53: 143-152.

- Berner, D. K., W. L. Bruckart, C. A. Cavin, J. L. Michael, M. L. Carter, and D. G. Luster. 2009. Best linear unbiased prediction of host range of the facultative parasite *Colletotrichum gloeosporioides* f. sp. *salsolae*, a potential biological control agent of Russian thistle. Biological Control 51:158-168.

- Berner, D. K., W. L. Bruckart, C. A. Cavin, and J. L. Michael. 2009. Mixed model analysis combining disease ratings and DNA sequences to determine host range of *Uromyces salsolae* for biological control of Russian thistle. Biological Control 49:68-76.

Using BLUPs to construct non-target test lists

Target →

Genus species	BLUP	S.E.
Rhaponticum repens	70.73	35.51
Arctium minus	-3.66	36.72
Callistephus chinensis	7.68	36.09
Centaurea montana	5.77	35.67
Plectocephalus rothrockii	0.79	36.05
Cichorium intybus	-3.93	36.15
Cirsium pitcheri	0.79	35.77
Crupina vulgaris	12.83	36.65
Erigeron rhizomatus	16.58	35.85
Helianthis eggertii	3.95	36.33
Helianthis schweini	4.34	36.33
Krigia montana	-11.46	36.09
Picnomon acarna	3.64	36.14
Saussurea alpine	4.80	36.74
Serratula coronata	14.06	36.54
Solidago shortii	11.46	36.06
Stokesia laevis	15.41	35.44
Carthamus tinctorius	15.86	36.06
Cynara scolymus	1.55	35.86
Plectocephalus americana	4.25	35.28
Carduus tenufloris	-6.45	35.75
Carduus thoermeri	-3.97	35.72
Centaurea calcitrapa	-11.42	35.79

🟨 🟦 = no data

🟦 = possible susceptible

- Iteratively test, re-analyze, re-test until suspect reactions clarified
- Iteratively include more closely related species and sequences in each test-and-analysis cycle

Is “what you see” what you get?

- Simple random sampling of test material assumes that the samples are truly representative of the species
- False positives and negatives, from a species perspective, are a real probability
- No indication of probability of “correct” evaluation

Is “what you see” what you get?

- In the case of CGS, each BLUP in our evaluation is based on 89 species’ interactions in addition to observed data
- This greatly increases the probability that the BLUPs are representative of the species
- Probabilities of taxon differences can be tested