Morphometric analysis of leaf variation in three North American grape species (*Vitis acerifolia, V. riparia*, and *V. rupestris*)

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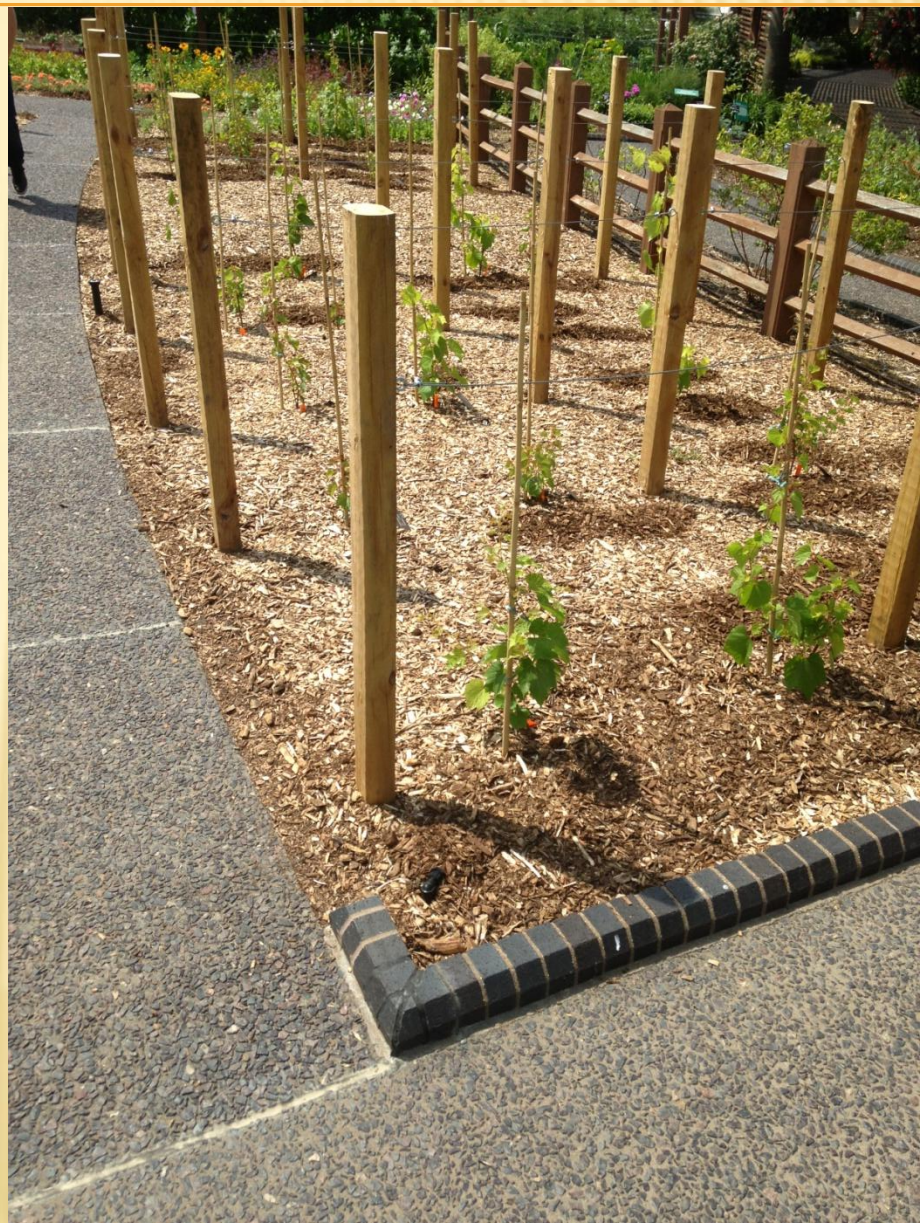
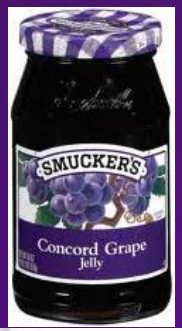
OVERVIEW

- Introduction
- Research Question
- Objective
- Methods
- Results
- Discussion
- Future Work



WHEN YOU THINK OF GRAPES, WHAT DO YOU THINK?...




WHAT I NOW THINK AFTER THIS SUMMER

INTRODUCTION

- Species within the genus *Vitis* (Vitaceae):
  - Foundation of industries:
    - Wine
    - Table grape
    - Raisin
    - Grape juice

- Although grapevine cultivation is based primarily on the **European grapevine *V. vinifera***, North American *Vitis* species play an important role.

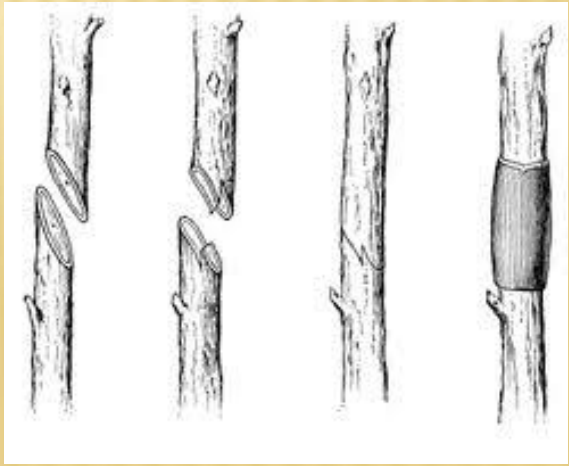
FAMILY: VITACEAE

- 12 genera:
  - Largest genus = *Cissus*
    - 350 species
  - *Vitis*
    - 60-70 species
- Other woody vines:
  - Boston ivy (*q.v.; Parthenocissus tricuspidata*)

  - Virginia creeper (*q.v.; P. quinquefolia*)

GRAPEVINES

- Unusual crops because cultivated varieties often consist of **two genetically distinct entities** that are grafted together:
- **Above**-ground part = <u>scion</u>
  - Produces the stem, leaves, flowers, and berries
- **Below**-ground part = <u>rootstock</u>
  - Forms the lower stem and roots



NOT A NEW IDEA

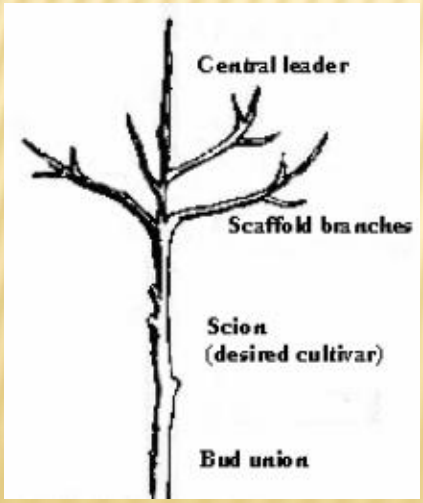
- Widespread grafting since the mid-1800's
  - Why?
    - Insects in the genus *Phylloxera* devastated the French grape industry.

    - North American grapevines have been key genetic resources for the development of:
      - Biotic and abiotic stress resistant rootstocks
      - Hybrid grape varieties

TODAY

- Vineyards in France (and other regions) consist of:
  - European *V. vinifera*

    grafted to
  - North American *Vitis* species



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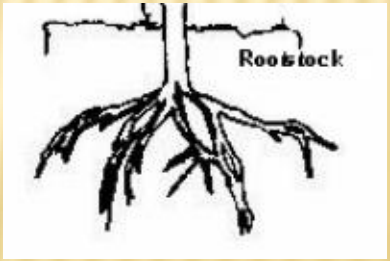

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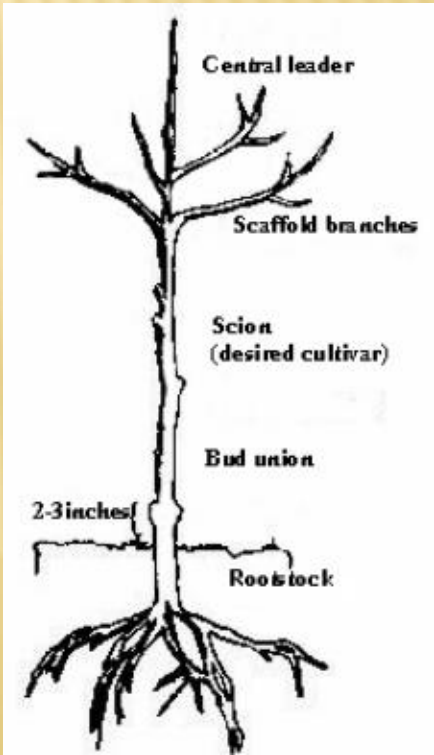


WE CAN **NOT** IGNORE THE **ROOT** OF THE PROBLEM…

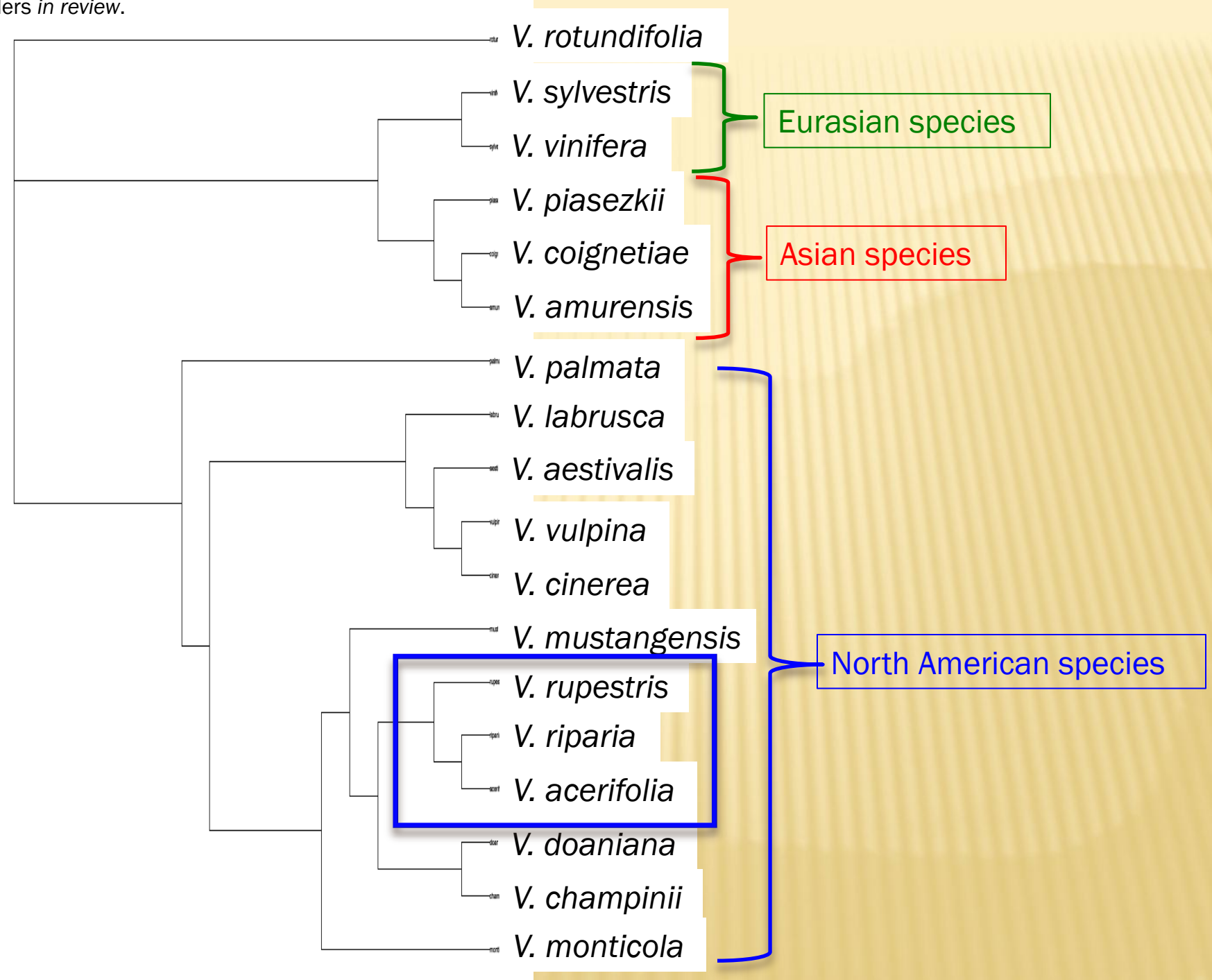
- Despite their importance for grape growing:
  - Neglect of morphological variation in natural populations of *Vitis* species used for rootstocks.

FOCAL SPECIES:

- Noth A

  - Bus *a)*
    - B reas
  - Rive
    - C
  - Rock grape (
    - Crawling in eds

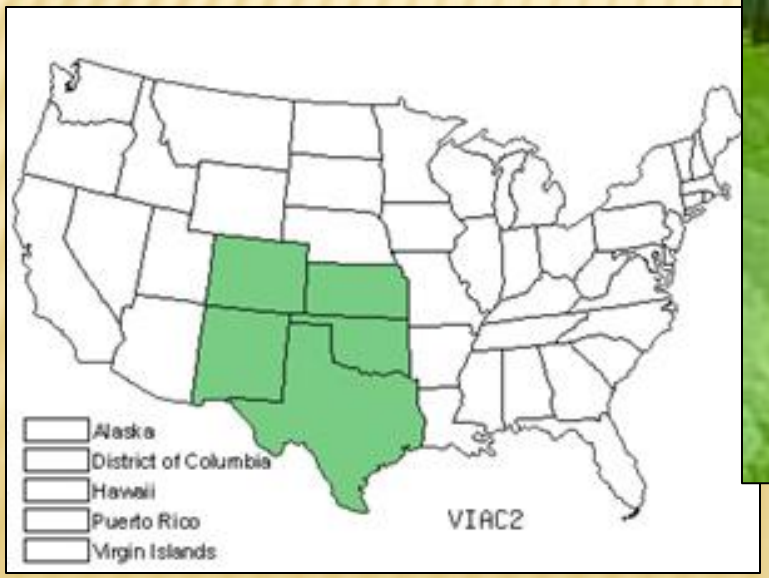
Miller/Mylers *in review*.



Vitis acerifolia

(Bush or Maple-Leaf)

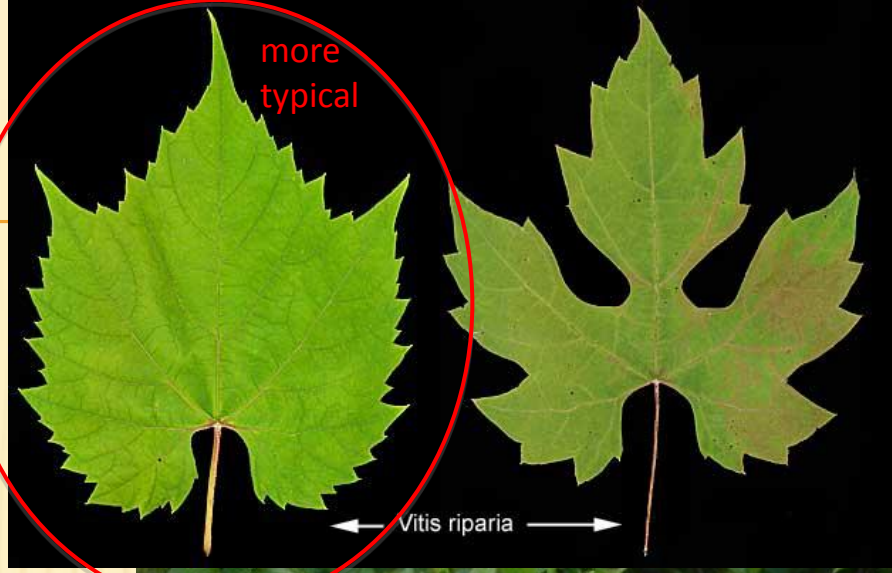
- Original name, *Vitis longii*
- Native to panhandle area
- Second in cold-hardiness among grapes only to *V. riparia*.

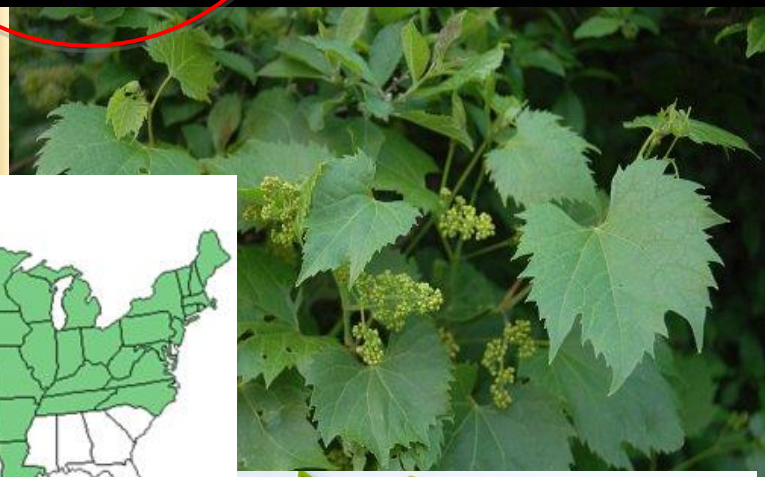


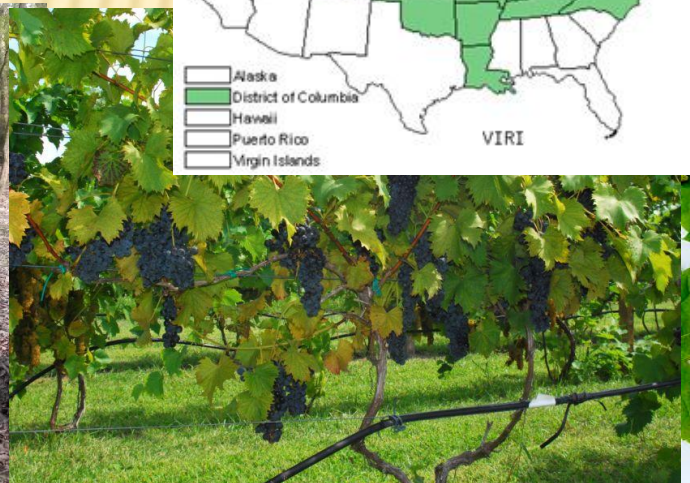
Vitis riparia

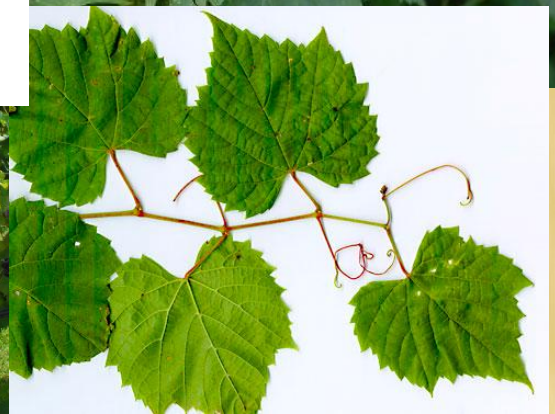
(River or Frost)

- Low woodlands near streams
- Heart-shaped leaves
- Forked tendrils
- Climbing vine
- Thick, woody vines with peeling, strip-like bark



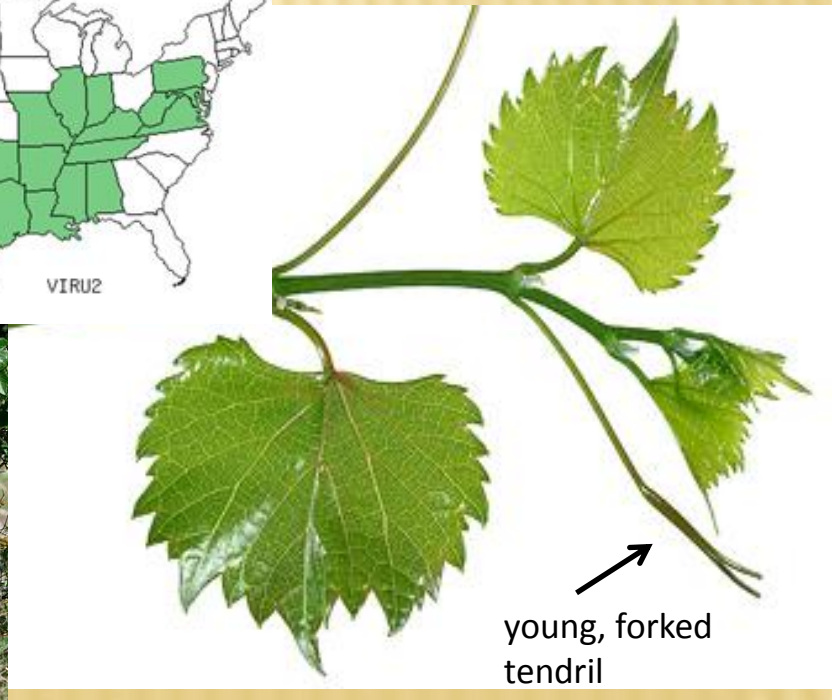






Vitis rupestris
(Rock)

- Rocky soils near streams/ dry creek beds
- Square/kidney-shaped leaves
- **Broader** than long
- Leaves often **conduplicate** (i.e., folding on itself)
- Young stem stalks often **red**
- Tendrils often absent (or forked)
- Srub-like growth habit; rarely climb



RESEARCH QUESTIONS

V. acerifolia, *V. riparia*, and *V. rupestris* differ in the environmental conditions subjected to:

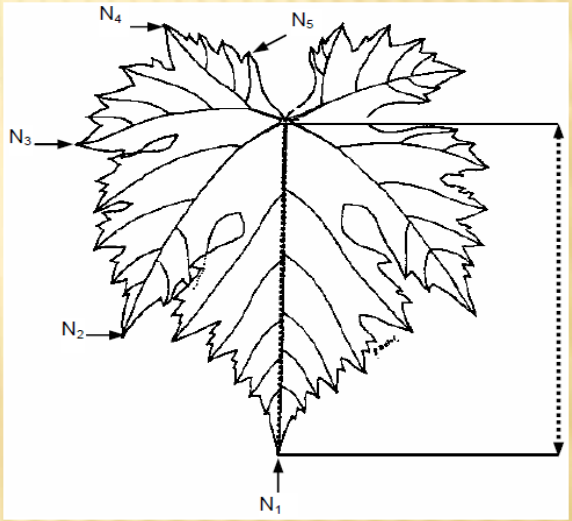
- Can these **differences in morphology** be quantified?
  - If so, can these measurements provide evidence for **species delimitation**?

OBJECTIVE

- The purpose of this study is to identify morphological traits that differ among three closely related *Vitis* species whose natural distributions differ with respect to abiotic conditions.

METHODS

- Determine Characters:
  - Standards-
    - What has been done in the past?



- What appears most different?
  - It is okay to go to the obvious
    - Leaves in *Vitis* species exhibit striking diversity (Galet 1979)

METHODS

- Apply comprehensive morphological indices developed by:
  - *The International Organization of Vine and Wine* (OIV)
  - *A Practical Ampelography: Grapevine Identification* (Galet 1979)

- In order to:
  - Characterize variation in **25 leaf characters** in at least **10 accessions** each of *V. acerifolia*, *V. riparia* and *V. rupestris*.

UNDERSTANDING THE DIFFERENCE….

- Living

- Dead

METHODS

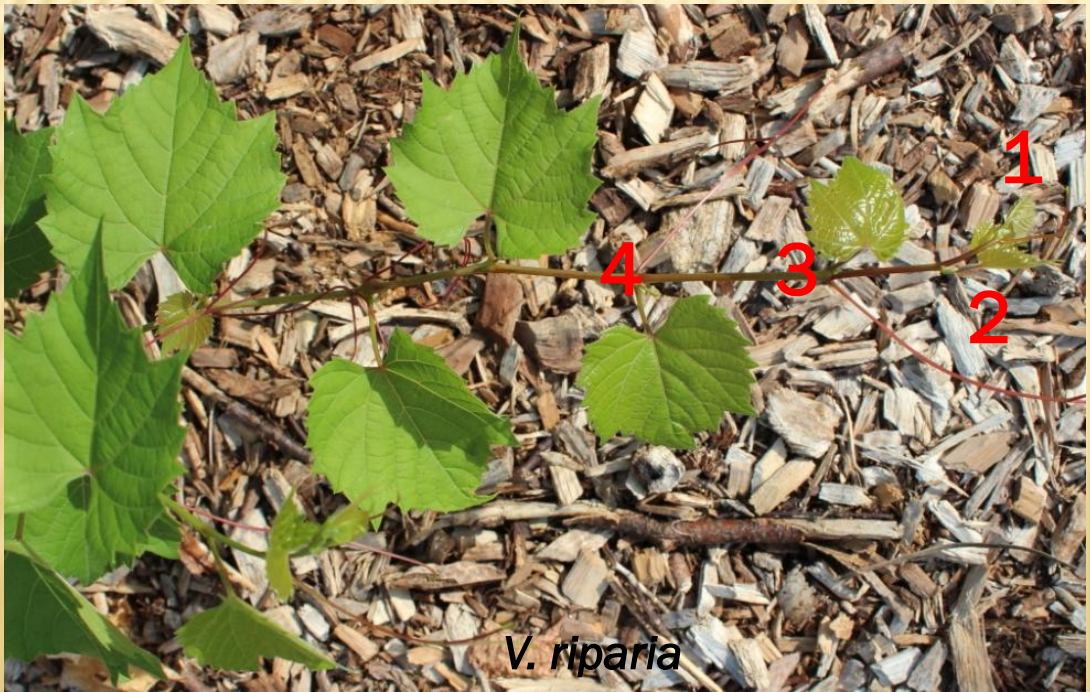
- Natural variation in leaf morphology is preserved through plant specimens housed in herbaria.
  - (*V. rupestris*)
    - 117 specimens
  - (*V. acerifolia*)
    - 24 specimens
  - (*V. riparia*)
    - 505 specimens

METHODS

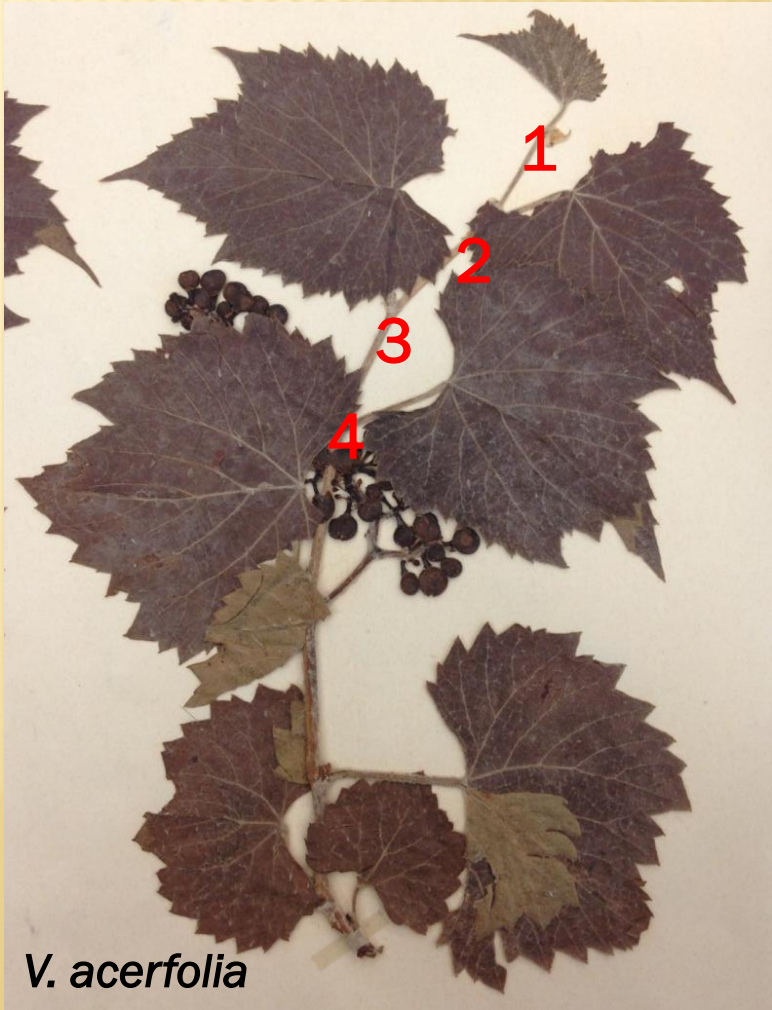
- Screen herbarium specimens
  - Diverse geographic locations
  - Complete

METHODS

- Locate the leaf to be measured:
  - Maturity
    - ~Fourth node down
      - Seen in nature!



V. riparia



V. acerfolia

EXAMPLES OF DIFFICULTIES

V. rupestris

V. riparia

METHODS

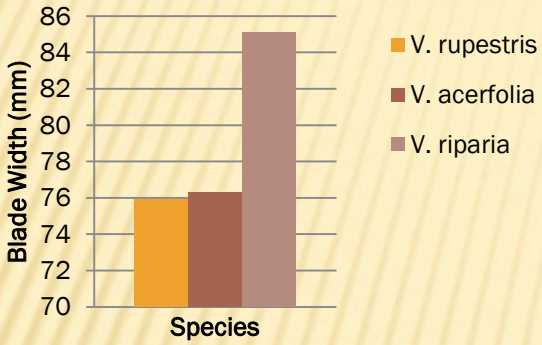
- Take measurements:

METHODS

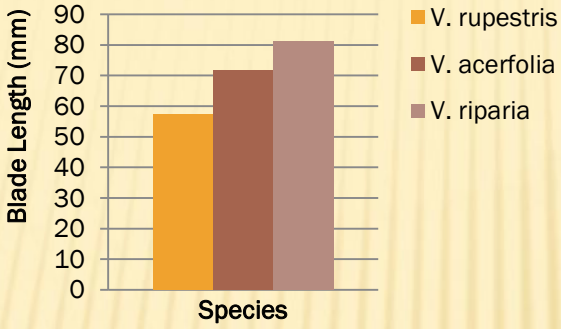
- Record and Organize Data:
  - +What makes sense?
  - +Format for R

Number	Blade.Width Blade.Shape TeethN4.Width	Blade.Length Blade.Size TeethN4.Length	Teeth.Margin Blade.Ratiolengthwidth Ratio.LengthpetioleN1	Petiolarsinus.Width Code.Ratiolengthwidth Length.N2	Petiolarsinus.Length Petiolarsinus.Ratiolengthwidth Length.N3	Petiolarsinus.Opening TeethN2.Ratiolengthwidth Length.N2 4	Petiolarsinus.Base TeethN4.Ratiolengthwidth Length.N5	Petiole.Length TeethN2.Width Species	Blade.Indument TeethN2.Length
1	74.31	59.53	3	36.56	11.11	1	2	30.96	0
	5	4423.6743	0.801103485	1	0.303884026	0.64368932	0.699724518	10.3	6.63
	7.26	5.08	0.520073912	48.3	39.11	22.49	7.51	rupestris	
2	83.97	51.93	3	47.67	7.24	1	2	59.74	0
	5	4360.5621	0.618435155	0	0.151877491	0.543646409	0.579055441	9.05	4.92
	9.74	5.64	1.150394762	50.67	43.6	25.75	9.86	rupestris	
3	[illegible]	[illegible]	62.61	3	30.47	9.48	1	2	51.91
	0	5	5069.5317	0.773249352	0	0.311125697	0.503827418	0.407284768	14.37
	7.2	9.06	3.69	0.829100783	54.61	41.93	20.84	5.67	rupestris
71.17	58[illegible]	3	47.91	6.95	1	2	36.19	0	5
	41[illegible]	[illegible]	1	0.145063661	0.721638655	0.593582888	9.52	6.87	7.48
	4.44	0.613701882	49.75	41.08	23.98	10.15	rupestris		
5	102.71	91.2	3	33.66	12.75	1	1	32	0
	4	9367.152	0.88793691	1	0.378787879	1.515151515	1.013793103	11.22	17
	7.25	7.35	0.350877193	82.74	48.48	27.7	10.22	rupestris	
6	65.2	49.33	3	21.35	8.31	1	1	65.75	0
	5	3216.316	0.756595092	0	0.389227166	0.658048374	0.587326121	11.99	7.89
	6.47	3.8	1.332860328	40.75	32.99	17.33	5.74	rupestris	
7	84.88	54.35	3	37.29	19.06	1	3	36.29	0
	5	4612.228	0.640315[illegible]	0	0.511129989	0.690647482	0.485875706	8.34	5.76
	7.08	3.45	0.967702292	48.46	33.45	27.8	13.02	rupestris	
8	4[illegible]	28.5[illegible]	3	3.26	8.9	1	1	25.68	0
	5	[illegible]19682	0.[illegible]174115[illegible]	[illegible]	[illegible]352338[illegible]	[illegible]	1.046831956	7	4.33
	3.63	3.8	0.898530441	28.2	24.7	15.76	6.58	rupestris	
9	60.13	46.51	3	33.28	4.65	1	2	18.97	0
	5	2796.6463	0.77349077	0	0.139723558	0.527486911	0.512867647	7.64	4.03
	5.44	2.79	0.[illegible]7869275	42.7	36.21	19.71	10.62	rupestris	
10	39.3	3[illegible]	3	[illegible]84	[illegible]76	1	2	28.07	0
	[illegible]	[illegible]05	0.7[illegible]7[illegible]	[illegible]	[illegible]291796	1.058536585	0.840425532	8.2	8.68
	3.46	[illegible]	0.[illegible]97[illegible]02	[illegible]	46.2	25.83	10.99	rupestris	
11	66.36	67.14	4	27.19	11.4	1	1	25.88	1
	2	4455.4104	1.011754069	3	0.419271791	1.022041763	0.772972973	8.62	8.81
	5.55	4.29	0.385463211	51.02	33.86	20.79	8.85	acerfolia	
12	98.05	87.37	4	39.87	14.11	1	2	26.39	1
	2	8566.6285	0.891075982	1	0.353900176	1.240317776	1.097238896	10.07	12.49
	3.33	[illegible]14	0.3[illegible]8758	71.85	53.84	31.68	15.2	acerfolia	
13	74.84	[illegible]6.0[illegible]	4	24.17	11.75	1	1	32.76	1
	2	[illegible]893[illegible]	[illegible]164[illegible]5061	3	0.486139843	0.865591398	0.707641196	9.3	8.05
	6.02	4.26	0.430655975	64.73	38.07	22.88	13.7	acerfolia	
14	74.8	85.96	4	37.25	5.68	1	2	27.05	1
	2	6429.808	1.149197861	4	0.152483221	1	0.553374233	11.43	11.43
	8.15	4.51	0.314681247	69.57	46.575	30.82	11.63	acerfolia	
15	88.72	76.92	4	18.02	20.49	1	1	29.47	1
	2	6824.3424	0.866997295	1	1.137069922	0.819105691	0.746478873	9.84	8.06
	11.36	8.48	0.383125325	54.71	42.66	27.18	11.53	acerfolia	
16	84.46	65.11	4	29.11	21.54	3	1	20.74	1
	2	5499.1906	0.770897466	0	0.739951907	0.952747253	0.68771527	9.1	8.67
	8.71	5.99	0.318537859	60.91	43.24	30.63	10.69	acerfolia	
17	73.67	69.74	4	24.7	13.02	3	1	24.45	1
	2	5137.7458	0.946653998	2	0.527125506	1.111547526	1.139130435	12.73	14.15
	4.6	5.24	0.350587898	54.05	38.96	22	12.82	acerfolia	
18	53.73	61.58	4	29.59	4.19	1	1	24.14	1
	2	3308.6934	1.146100875	4	0.141601893	0.761986301	0.829317269	5.84	4.45
	4.98	4.13	0.392010393	40.16	29.15	15.01	2.62	acerfolia	
19	85.77	76.52	4	14.39	17.83	3	1	39.94	1
	2	6563.1204	0.892153434	1	1.239054899	0.92704918	0.913528591	12.2	11.31
	7.17	6.55	0.521955044	59.6	42.4	24.2	14.99	acerfolia	
20	62.65	49.39	4	22.09	11.78	3	3	22.11	1
	2	3094.2835	0.788347965	0	0.533272974	0.535714286	0.474025974	8.4	4.5
	7.7	3.65	0.44766147	38.26	31.91	22.8	13.2	acerfolia	
21	69.7	71.39	4	9.64	13.26	3	1	28.53	0
	2	4975.883	1.024246772	3	1.375518672	1.07823741	0.704225352	11.12	11.99
	9.23	6.5	0.399635803	53.66	34.99	19.31	9.29	riparia	
22	59.68	60.42	4	38.31	5.02	1	1	35.47	0
	2	3605.8656	1.012399464	3	0.131036283	0.940996948	0.761437908	9.83	9.25
	6.12	4.66	0.587057266	46.64	35.12	17.32	10.42	riparia	
23	80.15	81.69	4	27.52	15.99	3	1	34.01	0
	2	6547.4535	1.019213974	3	0.581031977	1.313294798	0.603293413	8.65	11.36
	6.68	4.03	0.416330028	62.21	42.47	25.88	13.84	riparia	
24	98.69	104.84	4	31.24	21.58	3	1	44.09	0
	2	10346.6596	1.062316344	3	0.69078105	0.959770115	0.460543338	13.92	13.36
	7.73	3.56	0.420545593	76.98	49.52	29.25	16.05	riparia	
25	84.56	87.36	4	41.16	5.98	1	2	34.21	0
	2	7387.1616	1.033112583	3	0.145286686	0.679621849	0.421630094	9.52	6.47
	6.38	2.69	0.391597985	68.89	47.08	27.7	10.26	riparia	
26	106.87	108.08	4	43.64	18.74	1	1	44.65	0
	2	11550.5096	1.011322167	3	0.429422548	1.721682848	1.082177161	12.36	21.28
	9.37	10.14	0.413119911	88.85	58.9	34.99	16.7	riparia	
27	69.38	60.75	4	31.66	11.96	3	1	39.62	0
	2	4214.835	0.875612568	1	0.37776374	0.728239845	0.624870466	10.34	7.53
	9.65	6.03	0.65218107	47.32	34.95	20.42	7.94	riparia	
28	69.77	57.08	4	30.53	10.83	3	1	31.97	0
	2	3982.4716	0.818116669	1	0.354733049	0.87628866	0.76230661	7.76	6.8
	7.11	5.42	0.5600911	51.89	37.17	19.69	10.57	riparia	
29	120.62	110.17	4	27.76	13.98	3	1	53.33	0
	2	13288.7054	0.913364285	2	0.503602305	1.034776437	0.715646259	14.09	14.58
	7.35	5.26	0.484070074	90.4	78.64	35.4	18.47	riparia	
30	91.63	71.66	4	34.37	15.08	3	1	52.61	0
	2	6566.2058	0.782058278	0	0.438754728	0.88042588	0.782648402	12.21	10.75
	10.95	8.57	0.734161317	62.75	47.98	28.19	11.9	riparia	

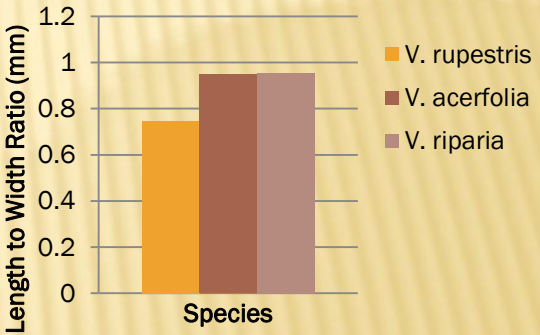
RESULTS



Blade Width



Blade Length



Ratio of Blade Length to Width

Beyond simply averages!

RESULTS

- By analyzing in **subsets**, a more **precise** comparison can be made.
- Consider sample size and dimensions analyzed.

Goals:

Describe leaf morphological variation in the three species

Identify traits that differentiate these close relatives

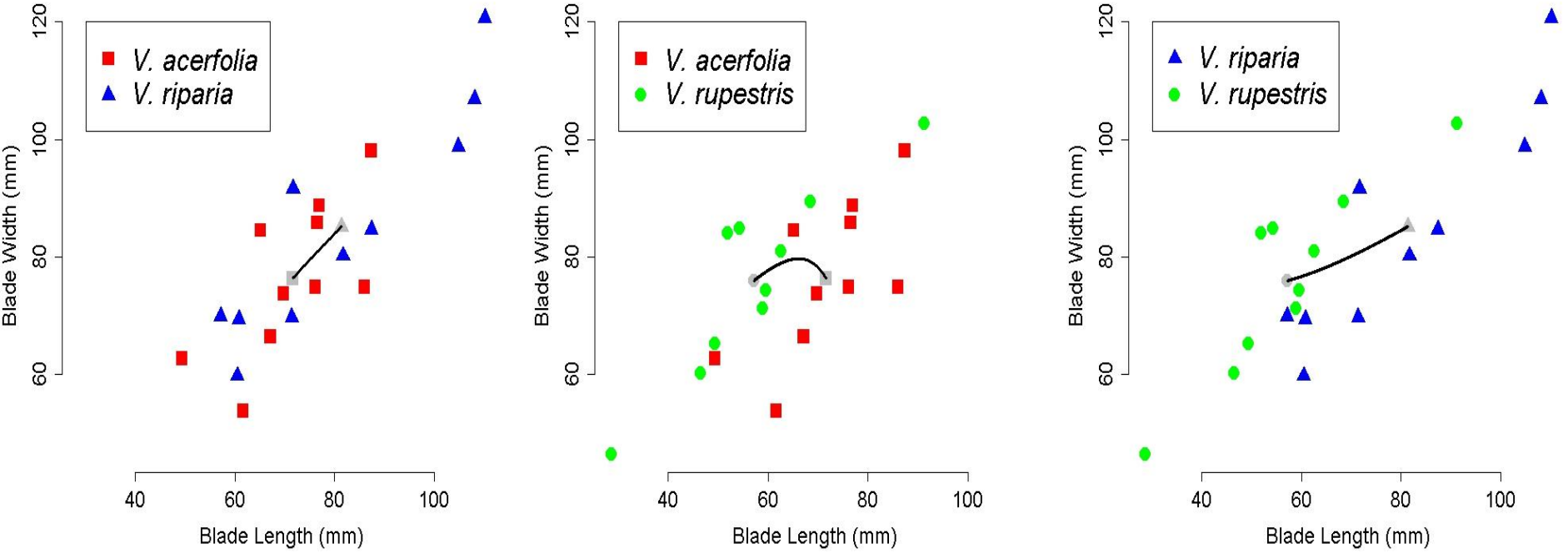


Figure 4:

- An R script was used to identify discontinuities (i.e., gaps) in morphological characters to test the hypothesis that three species of the genus *Vitis* are distinct lineages.
- A pairwise comparison using blade width and length characters was chosen as an example of visualizing a distribution of morphological variation.
- The ridgeline manifold travels between the mean (centroid, gray symbols) of each species.

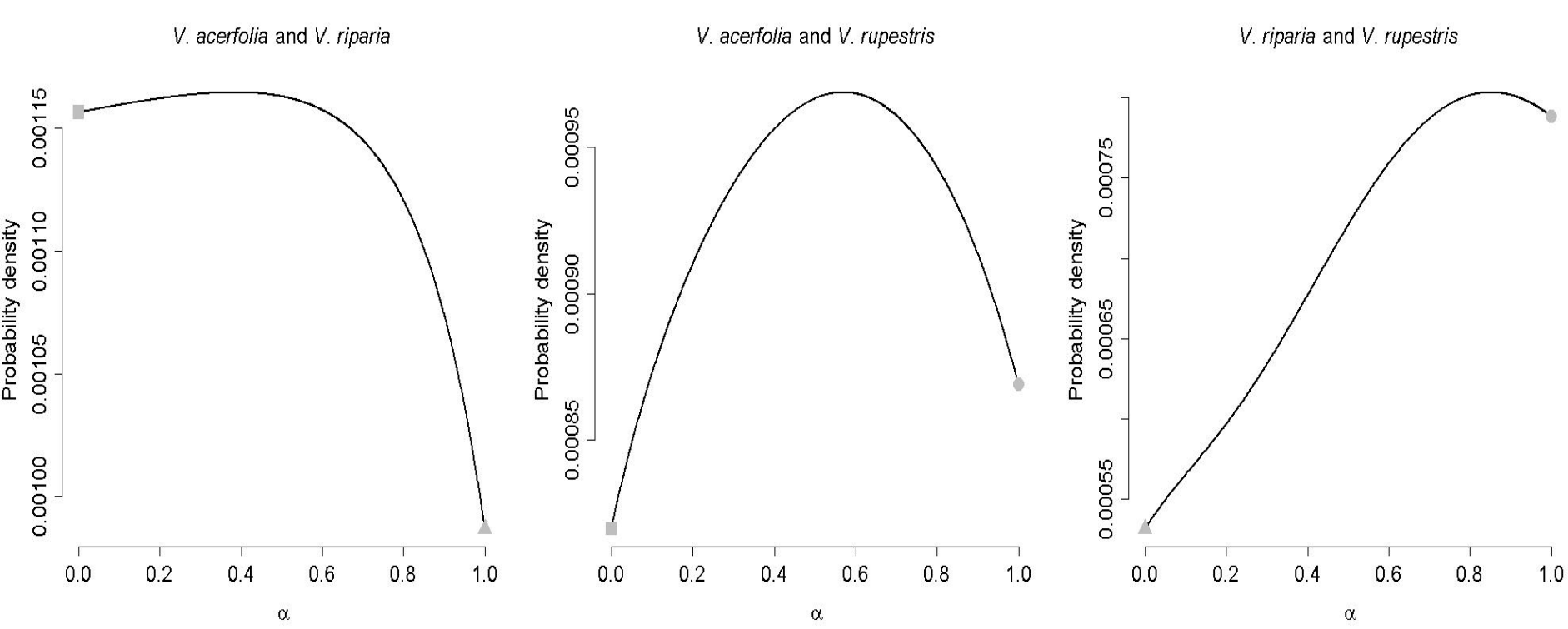
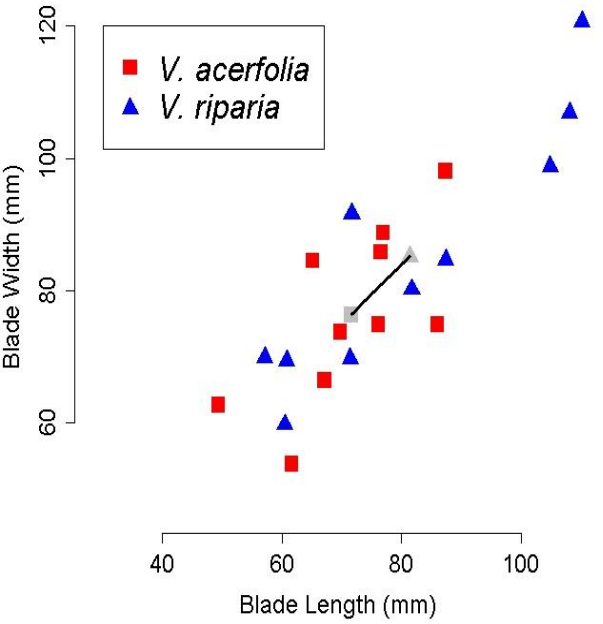
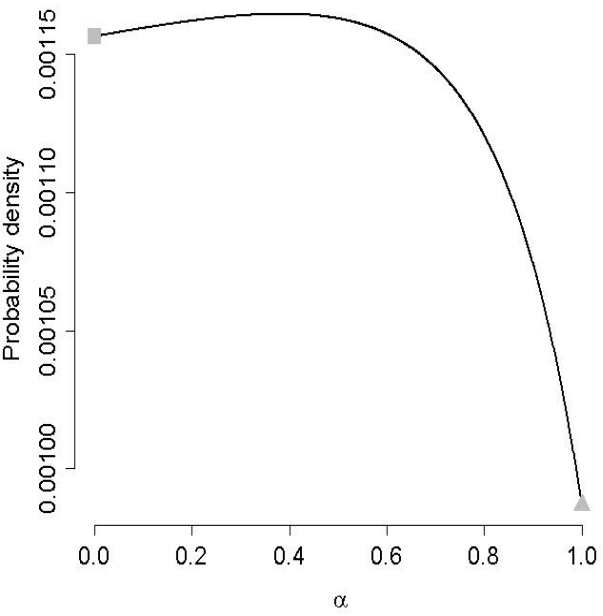


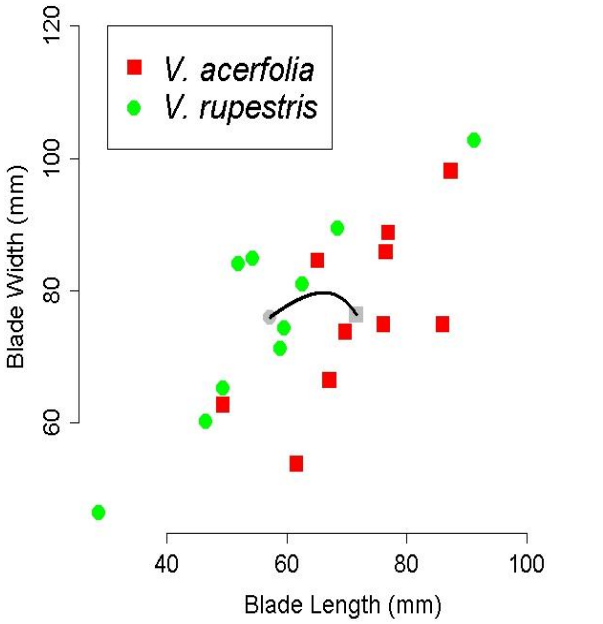
Figure 5:

- Distribution of the probability density function (a measure of the frequency of phenotypes) along the ridgeline manifold.
- Depressions in the probability density function of the ridgeline manifold equate to a morphological gap between two species.

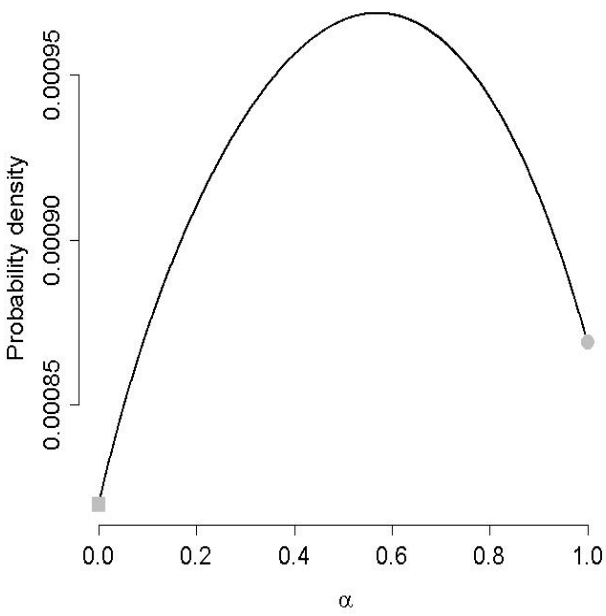


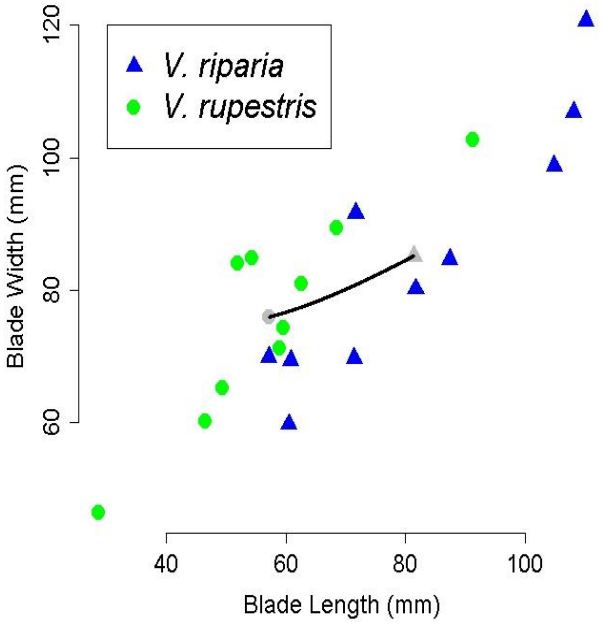
V. acerfolia and *V. riparia*



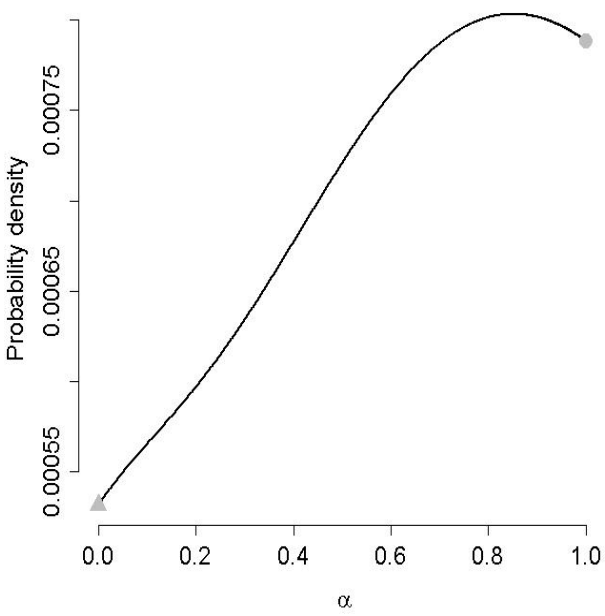


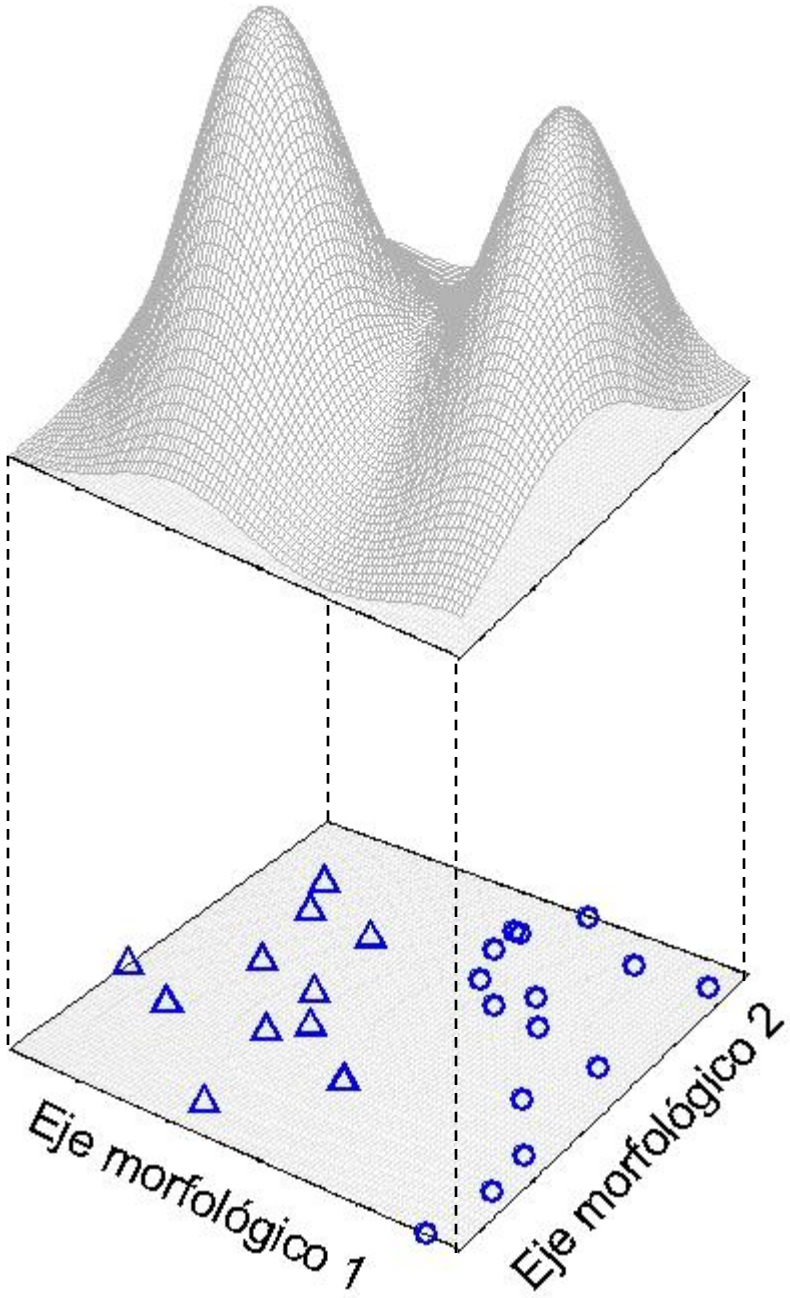
V. acerfolia and *V. rupestris*





V. riparia and *V. rupestris*

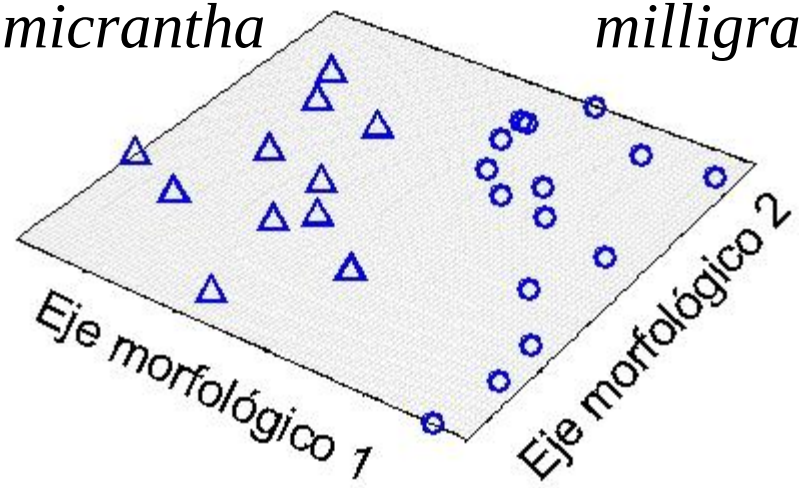




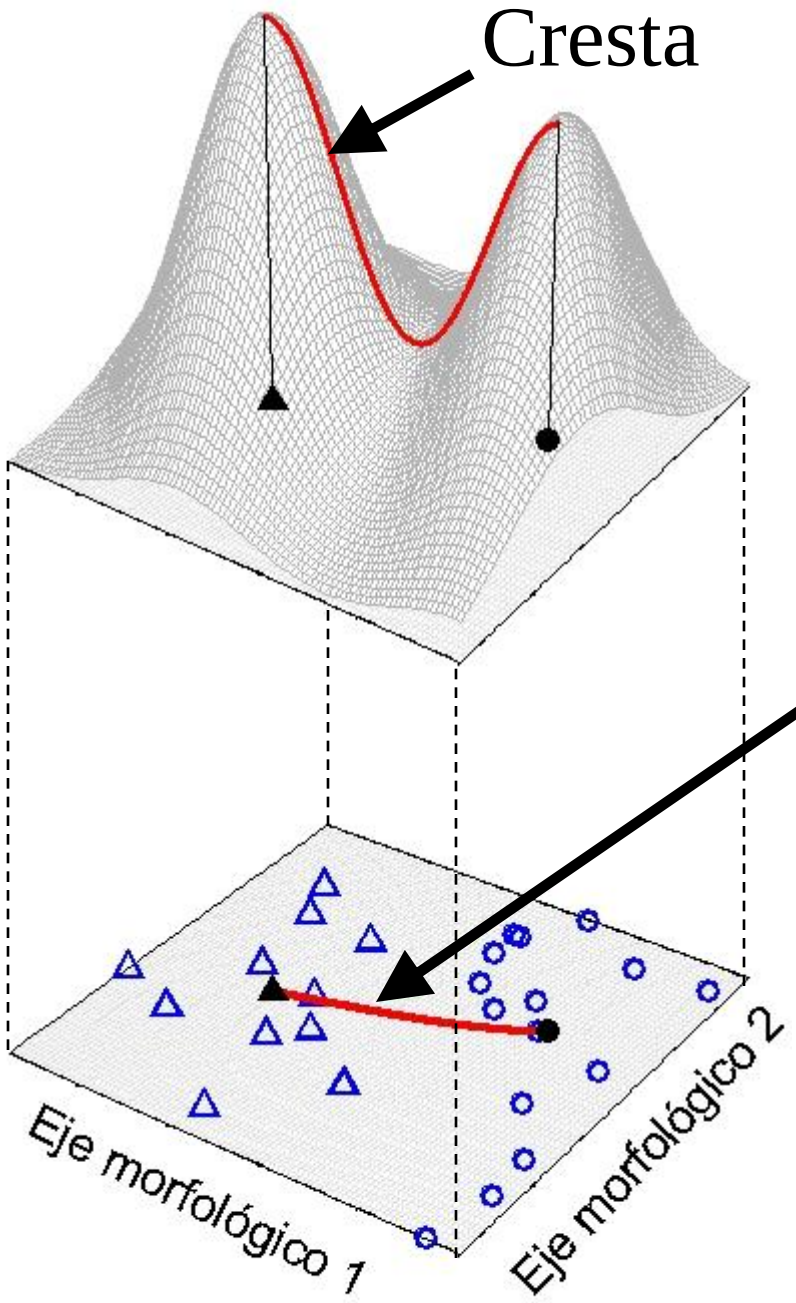
Jiménez & Zapata 2012

Escallonia micrantha

Escallonia milligrana

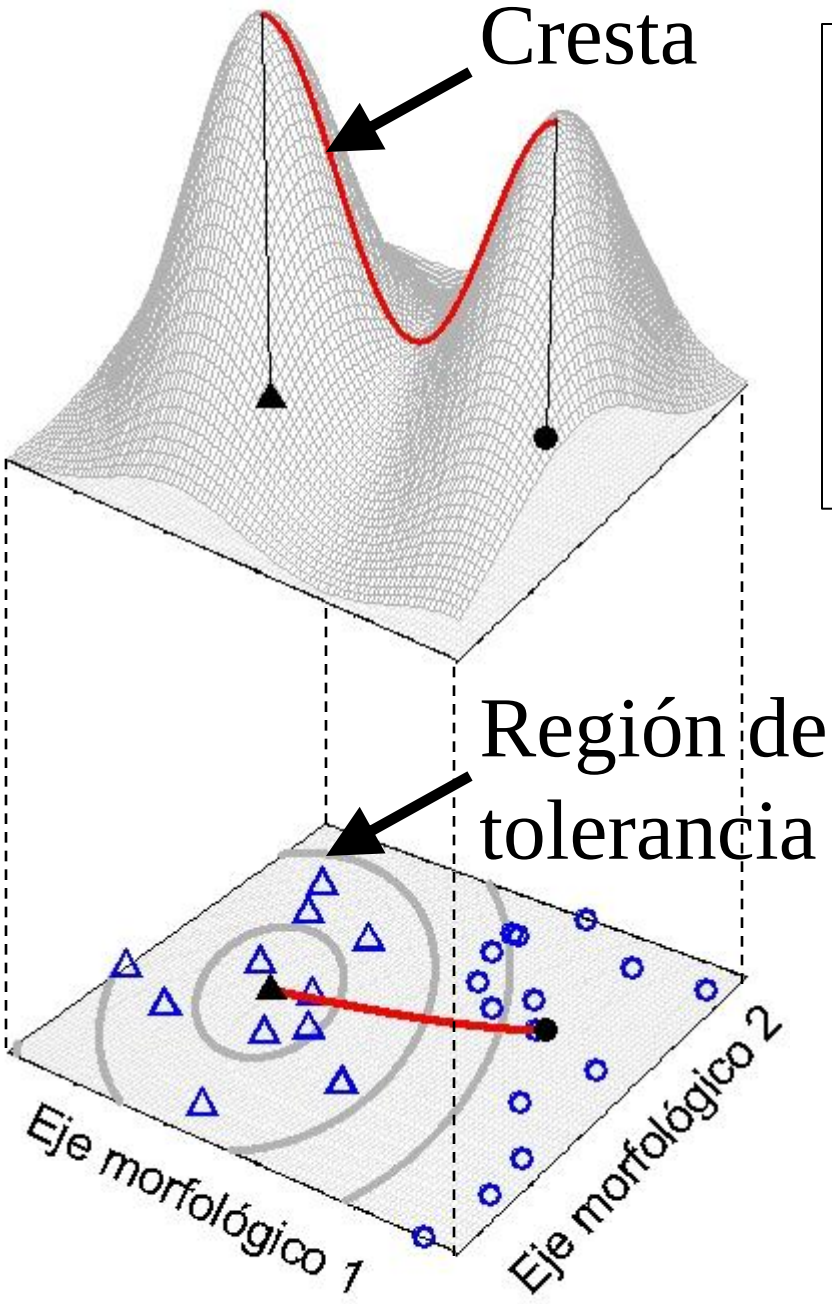


Jiménez & Zapata 2012



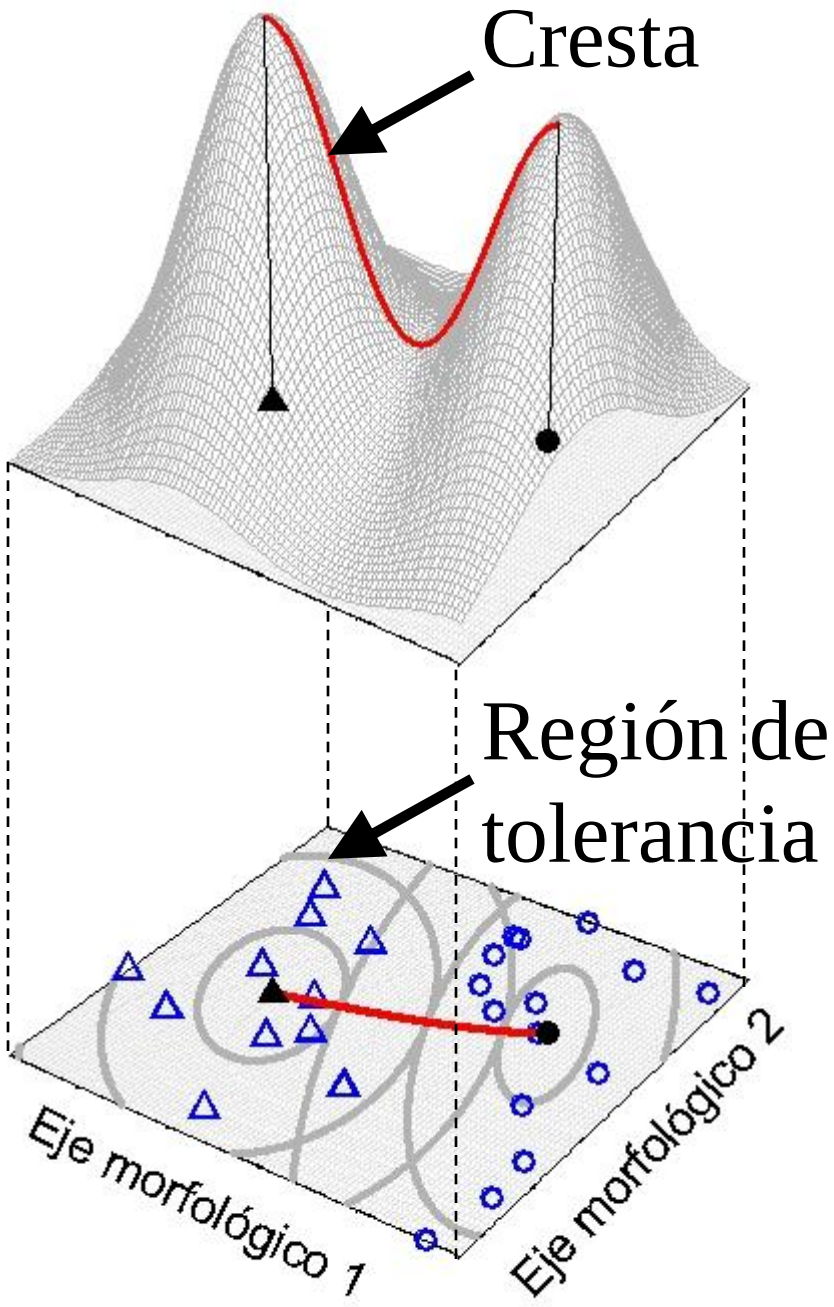
La cresta ("ridgeline manifold") incluye todos los puntos críticos de una mezcla de dos distribuciones normales multivariadas.
(Ray & Lindsay, 2005, Ann. Stat. 33:2042–2065).

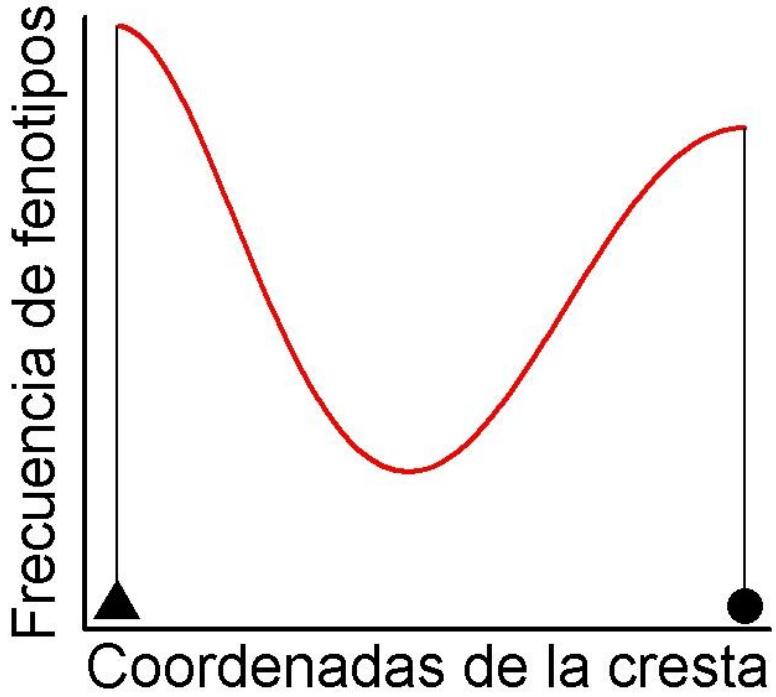
Jiménez & Zapata 2012

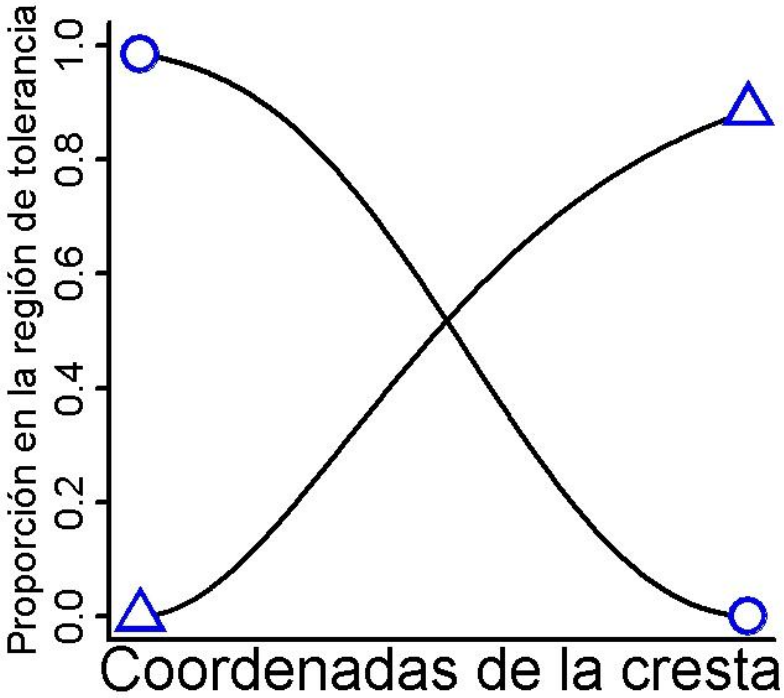


La región de tolerancia abarca una proporción de una distribución normal multivariada. (Krishnamoorthy & Mondal. 2006. Comm. Stat. Sim. Comp. 35: 461–478).

Jiménez & Zapata 2012







Jiménez & Zapata 2012

DISCUSSION

- Species are not “pulling apart” as much as one would think they would.
  - The probability density functions along the ridgeline manifold **shows no evidence** of a morphological gap for any of the species pairs.

DISCUSSION

- Although:
  - This conclusion is only for the morphological space defined by leaf blade length and width
    - May change with further sampling
- Illustrates that a difference between species in the means (or centroids) does not imply a gap (discontinuity).

DISCUSSION

- Way of describing natural variation associated with adaptation to dry environments in species used for rootstocks by the global grape industry.

- There are main characters that can be observed and measured that help classify species.

FUTURE WORK

- Modern morphometric techniques:
  - Digital phenotyping
    - Leaf imaging
      - Focus on quantifying **shape** variation

  1) Elliptical Fourier Descriptors (EFDs)

  2) Generalized Procrustes Analysis (GPA)
    - ImageJ
    - Cam2Com
    - ImageTool
    - R package analysis

LOOKING FORWARD

- What additional:
  - Traits can be measured?
  - Comparisons can be made?
- How do the traits observed relate to the environmental conditions of the area where they are located?

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REFERENCES

- Galet, P. 1979. A Practical Ampelography: Grapevine Identification. Comstock Publishing Associates a division of Cornell University Press, Ithaca and London.
- Zapata, F., and I. Jiménez. 2012. Species Delimitation: Inferring Gaps in Morphology across Geography. Systematic Biology 61(2): 179-194.
- 2009. 2nd Edition of the OIV Descriptor List for the Grape Varieties and *Vitis* Species. Organisation Internationale de la Vigne et du Vin.