

University of Colorado, Boulder

EBIO 4290 Phylogenetics Final Paper

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# Gall Wasps: Evolution of Gall Chambers in Association to the Location of Gall

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May 3, 2023

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EBIO 4290, Spring 2022, University of Colorado Boulder

## I. Introduction

There are varying examples of symbiosis between organisms in nature. However, it is interesting to see when nature provides the means for one organism to manipulate another to do its bidding, at no benefit to itself. Gall wasps are one such example. These organisms are insects classified in the family of Cynipidae. Gall wasps are known to induce gall structures in a variety of plant species by injecting their eggs into plant tissue. Through this process, the plant will grow an external structure, a gall, that serves as a protective layer for the growing larvae. In addition to being a protective layer, the gall is also utilized as a source of nutrients for the larvae [1].

The complexity of gall structure can vary depending on the host-plant species as well as the gall-inducing species [1]. Gall structure can be classified as cryptic, distinct swelling or complex. Galls that appear inside stems or twigs are denoted as cryptic galls, which do not induce external structures on the plant. Distinct swelling structured galls are external structures that appear to be similar to the normal structure of the plant, whereas complex galls are external but present themselves as extremely different from the host-plant. Moreover, galls can be categorized as integral or detachable, depending on how they are attached to the host. Integral galls will be sturdy on the host-plant, while detachable galls are easily removed. Another important feature of galls is that they can contain a single larval chamber or be multi-chambered. Multi-Chambered galls are interesting to examine since they are composed of several single larval chambers that aggregate to form

one, singular external gall. Lastly, galls may appear on several locations of the host-plant, which include: stems, twigs, runners, growing root tips, flowerheads, seed capsules, leaves, or buds. [2]

With the variety of gall structure, location, attachment, and chamber there are several opportunities for this species to show correlated discrete evolution among these characters. The two traits of interest in this study are the number of gall chambers and the location of the gall on the host. Gall wasps specifically feed on a layer of plant tissue lining the inside of the gall, unlike most other phytophagous insects [2]. Additionally, they are a short-lived species, and therefore only feed during the larval stage of growth. Moreover, they specifically depend on nutritional sources of sucrose and carbohydrates to maintain metabolism and increase survival [3]. Thus, it seems that the location of the gall on the host plant would provide different nutritious values to the larvae, but also influence the structural complexity of the gall itself. In turn, it seems that reproductive organs such as flower buds, flowerheads, and seed capsules would provide a more nutritious layer of plant tissue for the larvae.

To explore the correlations between the number of gall chambers and location of the gall, this proposed study will estimate a Bayesian phylogeny for the evolutionary history of gall wasps. Using methods of discrete correlation tests, such as Pagel tests, this study will investigate the relationship between these characters under the generated phylogeny. I hypothesize that galls present on the reproductive locations of a plant will be correlated with multi-chambered galls and on vegetative locations of the plant will present itself as single-chambered. Analysis of the

correlation between these traits can help us understand more about the developmental process that leads to the several, unique varieties of galls.

## **II. Materials and Methods**

### **II.1 Data Information**

All of the data collected is provided by Ronquist et. al from the paper *Evolution of Gall Wasp-Host Plant Association*. This paper scored 175 morphological characters for 41 taxa in a Maximum Parsimony framework. Using the exemplar tree built from this morphological data, Ronquist et al. created a meta tree for their trait mapping and estimations, which was limited to only the taxa that induce galls. Moreover, this paper provides character state information for the form, structure, position, chambers, and attachment traits of gall wasps which was collected by the researchers [2].

### **II.2 Phylogenetic Inference**

To generate a phylogenetic tree, the program MrBayes version 3.2.7a was used [4]. The dataset from the Ronquist et al. paper was a morphological character matrix scored for 175 characters. This matrix was in Nexus format; however, it was not MrBayes compatible due to the scoring of the characters. For morphological data inputs, MrBayes can only support a total of nine-character states that must be labeled with the numbers 0-9. The original morphological nexus data labeled the character states with the letters “a-p”, which thus prompted a necessary conversion. To accomplish this conversion, a python script (can be visualized in the GitHub repository link in the appendix) was written that would take each morphological character sequence and replace the most frequent character states with the numbers 0-9. The least frequent character states appeared at most 5

times over all sequences, and thus were replaced with a “-”. Following the conversion to MrBayes compatible Nexus file, the new morphology dataset was executed in MrBayes. The plan was to generate several high posterior probability trees, as well as a Bayesian consensus tree. Firstly, according to the exemplar tree from Ronquist et al. there were 4 outgroup taxa who do not induce galls. Therefore, before generating trees the outgroup taxon was set to *Ibalia rufipes*. Figure 7 in the appendix displays all taxa in the phylogeny and their classification. The data was then processed in MrBayes using an equal rates model as the evolutionary model, where rates will be sampled from the gamma distribution. Then, MCMC was performed over 2 runs with each run having 100,000 generations, sampling frequency at 100, and discarding the first 25% of runs. After the MCMC walk had completed, the convergence of the runs was verified through the PSRF values [4]. Finally, the resultant trees over the 2 runs were summarized using a measure of creating consensus of the posterior distribution using all clades compatible with each other. From this procedure, the highest posterior probabilities of all trees from the best log likelihood scored run were obtained, as well as consensus trees with strong posterior probability support for clades.

### **II.3 Comparative Methods**

After the generation of our phylogenies, comparative methods are necessary to assess correlation between the number of gall chambers and position of the gall. To run Pagel Tests (tests of dependency) [5] on the two traits, the software BayesTraitsV4 was used [6]. Primarily, two-character matrices needed to be built. One character matrix is required for creating a mirror tree of trait mapping in Mesquite version 3.7.0 [7]. The second character matrix is required for BayesTraitsV4 analysis. Using the character state data in the table provided by Ronquist et

al., a character matrix was constructed in Mesquite scoring values for the gall inducing taxa in the phylogeny. The scoring of the characters can be visualized in figure 5. Once the character matrix was built, a mirror tree window was created in Mesquite. The mirror tree window has parsimony traced character history for number of gall chambers on the left-hand side and traced character history for location of the gall on the right-hand side. The colors for the 4 states were coordinated in accordance to my hypothesis on both sides of the tree. Green was used to display single gall chambers and vegetative locations on the plant, while blue was used to display multiple gall chambers and reproductive locations on the plant [7]. The unscored taxa, which are either Inquilines (gall wasps that inject larvae into other existing galls) or non-gall inducing taxa, were left as gray since they should be unscored.

Using the character matrix that was developed in Mesquite, a new BayesTraitsV4 compatible character matrix was created using Visual Studio Code. To run the Pagel tests, two variations were performed. One variation was running the Omnibus test, Contingent Changes tests, and Temporal Order tests using the Bayesian consensus tree and the BayesTraits compatible character matrix. For all the tests mentioned below, they were performed using a Maximum Likelihood framework and chi-squared values were computed for the test by taking log likelihood difference, which resulted in obtaining p-values from the Vassar Stats website [6]. The omnibus test was conducted by running the dependent test in BayesTraits and then the independent test. These tests were executed with no constraints for the Omnibus test and setting constraints for Contingent Changes and Temporal Order tests. To understand the 8 rates of the dependent model and the constraints set, a graphic of the model can be seen in figure 4. The number of gall chambers was associated with the variable X

(state 0 = single, state 1 = multiple) and the variable Y was associated with the location of the gall (state 0 = vegetative, state 1 = reproductive). Looking at the 8-rate model in figure 4, for contingent changes the constraints set were  $q_{12}=q_{34}$  &  $q_{21}=q_{43}$ , as well as  $q_{13}=q_{24}$  &  $q_{31}=q_{24}$ . The first constraint will test if change in the state of gall location is dependent on number of galls and the second constraint will test if change in the number of galls is dependent on the state of gall location. For the temporal order tests, the constraints set were  $q_{12}=q_{13}$  and  $q_{42}=q_{43}$  [5]. The first constraint tests that the multiple gall chambers were gained before location of the gall and the second constraint tests that multiple gall chambers were lost before the location of the gall [5]. This process is repeated for the second variation which will use all resulting trees from the highest likelihood run of the MCMC walk. The only difference is that for this variation each test was logged to an output '.txt' file and then inputted into a Google Sheet where the average log likelihood of all trees, and minimum and maximum log likelihood over all trees is calculated.

### III. Results

#### III.1 Phylogenetic Inference

For the generation of the phylogenies, the results were successful. After running MCMC for 100,000 generations over 2 runs, the average standard deviation of split frequencies was 0.015831. MrBayes suggests that standard deviation should be less than 0.01, if it is not then additional generations need to be run [4]. However, looking at the additional results of the MCMC run below, we can see that the 2 runs do in fact converge. In the MrBayes log output, we can see that the two runs converge around an arithmetic mean of log likelihood score - 3071.50. The PSRF value for TL was noted as

1.000 and PSRF for alpha was 1.001. PSRF values should be 1.000 or extremely close to it, considering it is the measure of convergence of the two runs. Moreover, the average ESS (estimated sample size) was 497.7 for TL and 306.99 for alpha. MrBayes recommends that “ESS values below 100 may indicate that the parameter is undersampled”, which is not the case in these MCMC runs [4]. Out of 1407 trees samples, 50% credible set contains 657 trees, 90% credible set contains 1257 trees, 95% credible set contains 1332 trees, and 99% credible set contains 1392 trees. The result of this analysis showed that the likelihood score of MCMC run 1 was better than the likelihood of run 2 by 0.34. From run 1, we gained 1407 trees with one tree having the posterior probability of 1.0. The entire log file for the MrBayes computations can be found in the GitHub link in the appendix. The consensus tree, in figure 1 of the appendix, showed extremely strong posterior probability support for internal nodes, 95% PP support for the clade that contains all taxa between *Aylax papaveris* and *Andricus quercusradicis*. All nodes in this branch were generally about 90% PP except for 4 out of 15 nodes. MrBayes accurately generated this consensus tree phylogeny as it is almost identical to the exemplar tree by Ronquist et al. and maintains the same relationships of common ancestry. Taking the statistical measures of convergence of the 2 MCMC runs, as well as the consensus tree topology and posterior probability support, the phylogenetic inference seems to be reliable and statistically significant.

### III.2 Comparative Methods

The results of the comparative methods indicate that my hypothesis cannot be well supported. First, if we look at the mirror tree in figure 2, we can see that there does not appear to be much overlap between the correlations of the two traits that were proposed in my hypothesis.

We do not see extremely convincing evidence of correlation between single gall chambers and vegetative locations, nor multiple gall chambers and reproductive locations. To convince myself of the results, further Pagel tests were conducted and all results can be visualized in the table of figure 3. Running the Omnibus test on the Bayesian consensus tree and BayesTraits compatible character matrix resulted in a log likelihood score (abbreviated as  $\ln(L)$  from here on out) for the independent model being -36.527. The dependent model had an  $\ln(L)$  of -34.621. This resulted in a chi-squared value of 3.812 with 4 degrees of freedom, which evaluates to a p-value of 0.432. This p-value is not less than 0.05 and therefore not significant enough for us to choose the dependent model over the independent model. However, further Pagel tests were conducted to see if constrained dependent models would be significantly preferred over independent models, implying that there is correlation. For the Contingent Changes test, the constrained model for  $q_{12}=q_{34}$  &  $q_{21}=q_{43}$  had a  $\ln(L)$  of -34.623. This performed worse in likelihood than the dependent model with no constraints, and therefore was immediately rejected. The constrained model with  $q_{13}=q_{24}$  &  $q_{31}=q_{42}$  had an  $\ln(L)$  = -34.627, which again was a worse likelihood score and immediately rejected. Thus, the hypothesis must be rejected because we do not see change in either gall chambers or location of the gall being dependent on the state of the other. However, Temporal Order tests were still conducted and the constrained model with  $q_{12}=q_{13}$  had  $\ln(L)$  = -34.490 and the constrained model with  $q_{42}=q_{43}$  resulted in  $\ln(L)$  = -34.621. While the constrained model of gain had slightly better log likelihood, by 0.2, this is not enough to prompt p-value evaluation. Thus, both Temporal Order tests were rejected as well. An identical evaluation occurred when running BayesTraits with all 1407 trees from MCMC run 1 and the results can be seen in the

table of figure 6. The Omnibus test resulted in an average  $\ln(L)$  for the dependent model being -34.21 and the average  $\ln(L)$  for the independent model was -36.466. The difference in likelihood scores is almost identical to the difference in likelihood scores for the consensus tree. For the Contingent Changes and Temporal Order tests, similar insignificant p-values were observed and therefore the possibility that 1407 Bayesian trees could display a dependent relationship between the two traits was rejected.

#### IV. Discussion

As we can see from the results section, the hypothesis of this study must be rejected. Originally, the thought process was that the galls induced by gall wasps are dependent on the nutrients that are supplied to the gall. It was thought that reproductive structures provide more carbohydrates and nutrients to the plant-host tissue lining the gall, therefore leading to the evolution of multiple chambered galls. Moreover, it was expected that galls on twigs and stems (vegetative locations) would provide less nutrients and thus maintain the state of the single gall chamber. This was not the case, instead there does not appear to be correlation between the number of chambers in a gall and the location of the gall. Looking at the mirror tree again, we can see that for some taxa, especially the taxa in the Pha-Tim Complex family show signs of correlation between the single chambered galls and vegetative locations. However, this could be attributed to the fact that the taxa in the Pha-Tim Complex generally induce galls on stems and leaves, vegetative locations and can vary in number of chambers of gall [2]. It could be a pure chance that we can see the correlation for this clade. Moreover, for the rest of the phylogeny there does not seem to be correlation between the states of the two traits. Additionally, this pattern is not seen in other studies on gall wasps.

One of the key issues in this analysis could be scoring of the characters. To perform a proper analysis of this correlation the hypothesis should be framed as such: single chambered galls will be correlated with gall locations on stems, twigs, runners, growing root tips, and leaves while the multi-chambered galls will be correlated with gall locations on inflorescence, flower heads, buds, bract, seed, or seed capsule. Moreover, some of the taxa present themselves with the capabilities of producing single and multiple chambered galls so this would need to be accounted for as well. Thus, instead of binary character mappings for both taxa we would need to incorporate 3 states for the number of gall chambers and 12 states for the location of the gall. Using this method, there is a greater probability that we would see some sort of correlation between the number of gall chambers and position of the gall. However, this would require more time and large amounts computation power. Using this hypothesis, the dependent model would have 72 rates which is extremely complicated to work with and highly complex. A possible solution is to create 12 different character matrices for each gall location while maintaining the 3 states for the number of gall chambers. This method would allow for binary scoring of each location of the gall i.e., if location of the gall is stems or not. If we follow through with this method, there is a possibility we see some dependent relationships due to less complex dependent rate models, however this process would be very time consuming.

Additionally, another fault of my study could be the number of taxa sampled. With 41 taxa sampled and only 33 of these taxa being gall-inducing, there is not much phylogenetic signal in my data. 33 taxa do not seem to be adequate for visualizations of correlations between traits. Perhaps with supplementary taxa there could be phylogenetic signal present in the data.

While there does not seem to be correlation between the number of gall chambers and location of the gall in this study, there is still a possibility that this correlation can be explored with different methods in future work. Furthermore, there are other traits of galls that can be explored for correlation. For example, it could be investigated whether there is a correlation between the structural complexity of the gall and the location of the gall, or whether structural complexity of the gall is dependent on the number of gall chambers. There are several analyses that can be conducted in the future to learn more about galls and gall wasps, especially analyses related to the Inquilines who do not induce galls but rather embed their eggs into existing galls. This study excluded Inquilines in the comparative methods analyses since they do not produce galls. However, there is not much research done in the field of Inquilines, which prompts investigations into what adaptive selective pressures led to the evolution of this behavior of Inquilines.

Overall, research into Cynipidae and the variation of the galls that they produce needs to be further investigated. The work presented in this study was a simple investigation for relationships of dependency between gall traits that would be expected to have correlation. But the importance of this work is to assist in the understanding of how gall wasps can produce such a variety of galls, and further to understand the environmental phenomena of host-plant manipulations controlled by Cynipidae. It has been noted in previous studies that the differences in gall character traits have led to confusion in the classification of Cynipidae. The gall wasps have been classified as different species and even different genera [8]. Therefore, understanding the characteristics of galls and how they are interrelated, could provide clarity to classifications, and improve public knowledge of gall wasps.

## V. Literature Cited

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 Evolution, vol. 76, no. 8, 2022, pp. 1849–1867.,  
<https://doi.org/10.1111/evo.14562>.

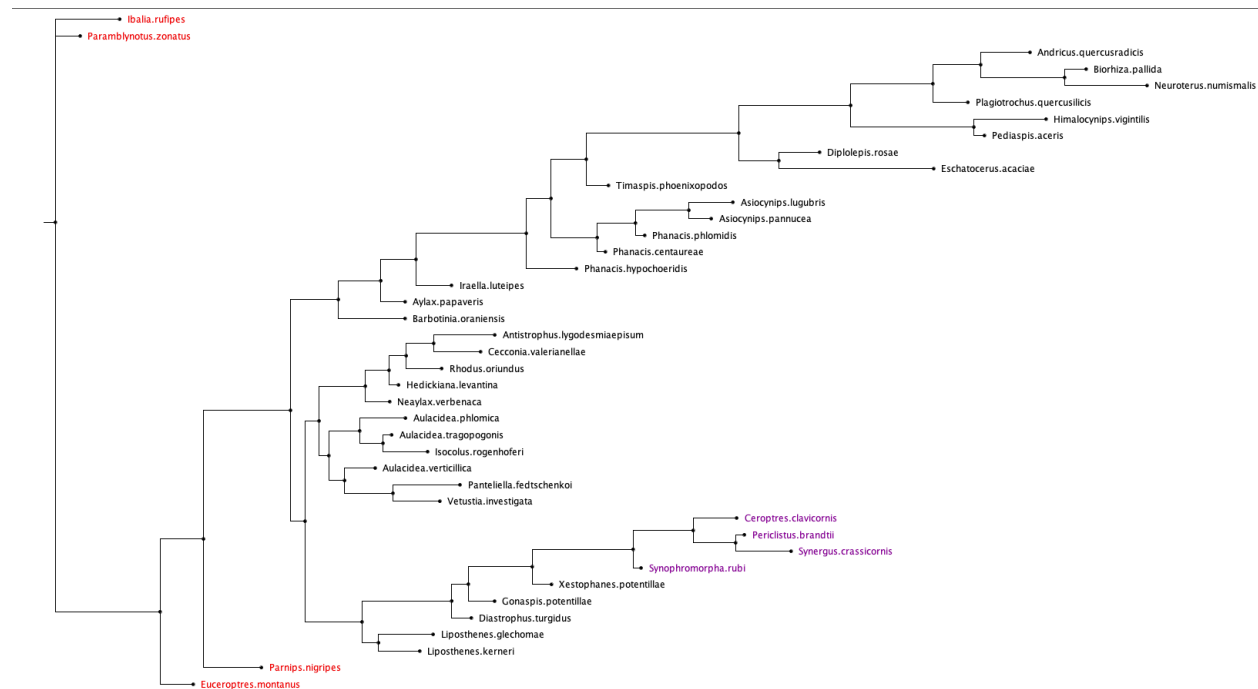
## VI. Appendix

Github Repository:

<https://github.com/ssarthak01/GallWaspsDiscreteTraitEvolution>

Figures listed on the following pages.

*Figure 1 - Bayesian consensus tree created from MrBayes using processed morphological data (red labelled taxa are outgroup and purple labelled taxa are Inquilines).*



*Figure 2 – Mesquite mirror tree with mapped traits built from character matrix and the Bayesian consensus tree (single chamber and vegetative locations highlighted in green; multi-chambered and reproductive locations highlighted in blue)*



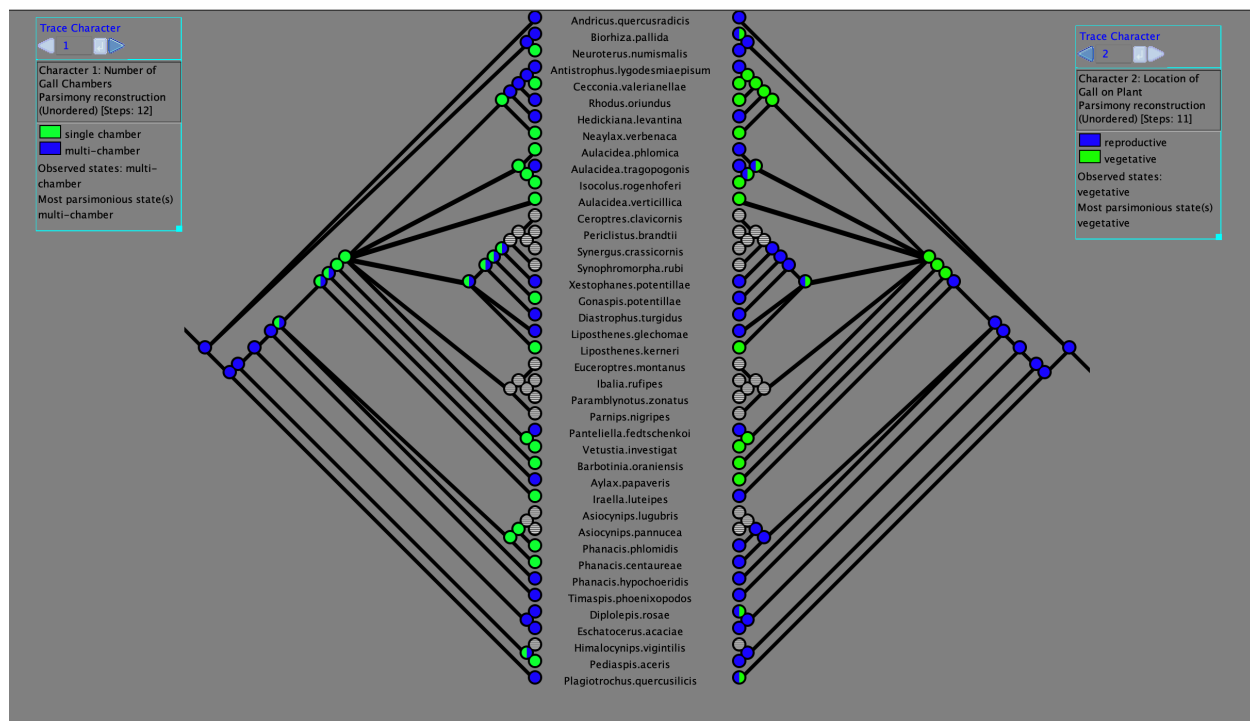


Figure 3 - Table of Pagel Test Results for Bayesian Consensus Tree with BayesTraitsV4 compatible character matrix (running: Omnibus test, 2 Contingent Changes Tests, and 2 Temporal Order Tests)

	Pagel Tests	Log Likelihood	X <sup>2</sup>	P-val
<b>Omnibus</b>	<b>Independent Model (df=4)</b>	-36.527	n/a	n/a
<b>Omnibus</b>	<b>Dependent Model (df=8)</b>	-34.621	3.812	0.432
<b>Contingent Changes</b>	<b>Dependent Model w/ constraints: q12=q34 &amp; q21=q43 (df=7)</b>	-34.623	0.004	0.9496
<b>Contingent Changes</b>	<b>Dependent Model w/ constraints: q13=q24 &amp; q31=q42 (df=7)</b>	-34.627	0.012	0.9128
<b>Temporal Order</b>	<b>Dependent</b>	-34.490	0.262	0.6087

	<b>Model w/ constraints: q12=q13(df=7)</b>			
<b>Temporal Order</b>	<b>Dependent Model w/ constraints: q42=q43 (df=7)</b>	-34.621	0.0	1.0

Figure 4 - Visualization of 8-rate dependent model used to set constraints for Pagel tests.

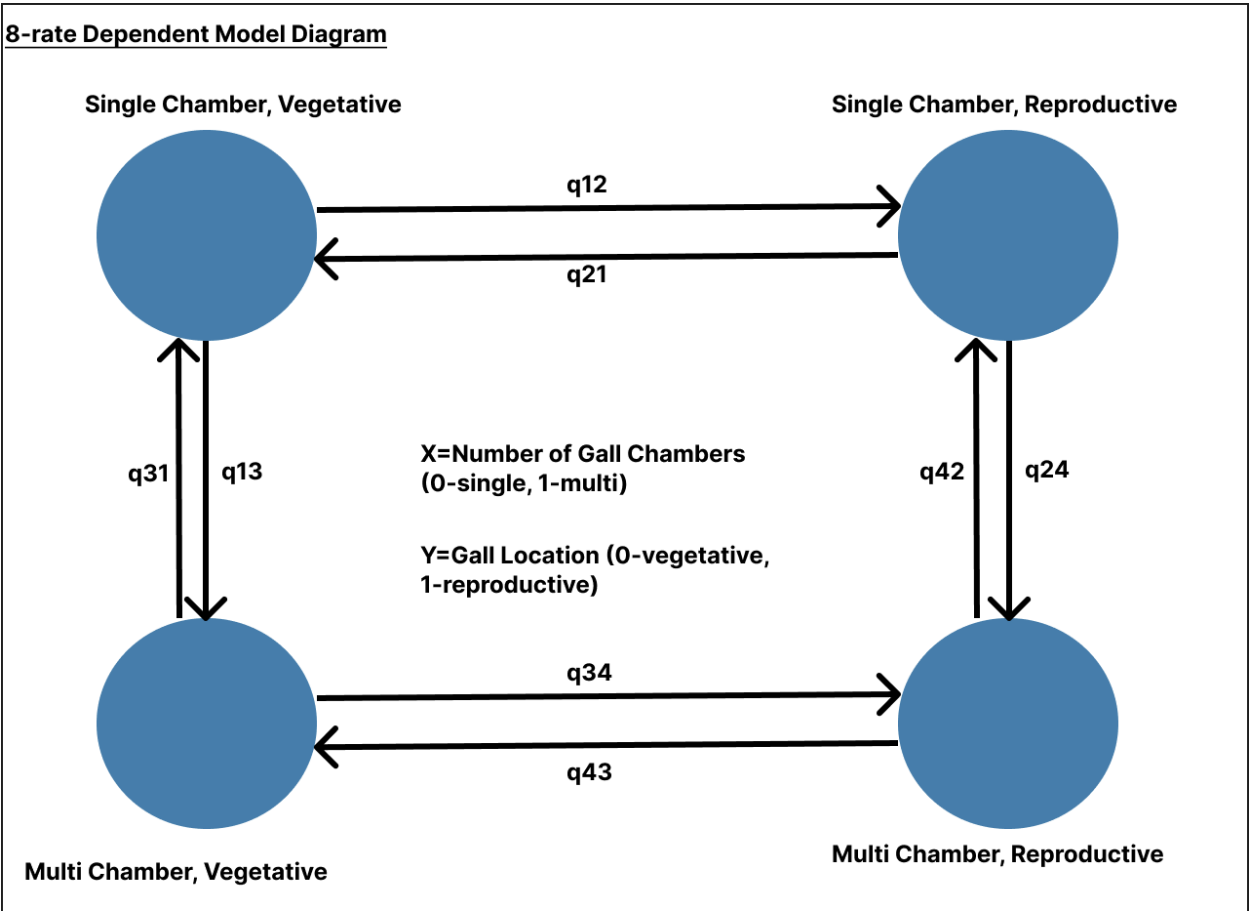


Figure 5 - Character matrix created in Mesquite and used to generate mirror tree.

Taxon \ Character		1	2
		Number of Call Charms	Location of Call on Plant
1	Ibalia.rufipes	–	–
2	Andricus.quercusradi	Many	Vegetative
3	Antistrophus.lygodes	Many	Vegetative
4	Asiocynips.lugubris	–	–
5	Asiocynips.pannucea	–	–
6	Aulacidea.phlomica	Single	Vegetative
7	Aulacidea.tragopogon	Many	Vegetative
8	Aulacidea.verticillica	Single	Reproductive
9	Aylax.papaveris	Many	Reproductive
10	Barbotinia.oraniensis	Single	Reproductive
11	Biorhiza.pallida	Many	Vegetative&Reproductive
12	Cecconia.valerianella	Single	Reproductive
13	Ceroptres.clavicornis	–	–
14	Diastrophus.turgidus	Many	Vegetative
15	Diplolepis.rosae	Many	Vegetative&Reproductive
16	Eschatocerus.acaciae	Many	Vegetative
17	Euceroptres.montanu	–	–
18	Gonaspis.potentillae	Single	Vegetative
19	Hedickiana.levantina	Many	Vegetative
20	Himalocynips.vigintili	–	–
21	Iraella.luteipes	Single	Vegetative
22	Isocolus.rogenhoferi	Single	Reproductive
23	Liposthenes.glechoma	Many	Vegetative
24	Liposthenes.kerneri	Single	Reproductive
25	Neaylax.verbenaca	Single	Reproductive
26	Neuroterus.numismal	Single	Vegetative
27	Panteliella.fedtschen	Many	Vegetative
28	Paramblynotus.zonat	–	–
29	Parnips.nigripes	–	–
30	Pediaspis.aceris	Single	Vegetative
31	Periclistus.brandtii	–	–
32	Phanacis.centaureae	Single	Vegetative
33	Phanacis.hypochoerid	Many	Vegetative
34	Phanacis.phlomidis	Single	Vegetative
35	Plagiotrochus.quercus	Many	Vegetative&Reproductive
36	Rhodus.oriundus	Many	Reproductive
37	Synergus.crassicornis	–	–
38	Synophromorpha.rubi	–	–
39	Timaspis.phoenixopo	Many	Vegetative
40	Vetustia.investigat	Single	Reproductive
41	Xestophanes.potentil	Many	Vegetative

Figure 6 - Table of Pagel Test Results for 1407 Bayesian Trees with BayesTraitsV4 compatible character matrix (running: Omnibus test, 2 Contingent Changes Tests, and 2 Temporal Order Tests)

	Pagel tests	Min to Max Log Likelihood Range	Average Log Likelihood	X <sup>2</sup>	P-val
Omnibus	Independent Model (df=4)	[-36.53, -34.09]	-36.47	n/a	n/a

<b>Omnibus</b>	<b>Dependent Model (df=8)</b>	[-34.67, -31.25]	-34.21	4.52	0.3402
<b>Contingent Changes</b>	<b>Dependent Model w/ constraints: q12=q34 &amp; q21=q43 (df=7)</b>	[-34.72, -31.62]	-34.52	0.202	0.6531
<b>Contingent Changes</b>	<b>Dependent Model w/ constraints: q13=q24 &amp; q31=q42 (df=7)</b>	[-34.74, -31.95]	-34.51	0.222	0.6375
<b>Temporal Order</b>	<b>Dependent Model w/ constraints: q12=q13 (df=7)</b>	[-34.69, -32.58]	-34.48	0.282	0.5954
<b>Temporal Order</b>	<b>Dependent Model w/ constraints: q42=q43 (df=7)</b>	[-34.67, -31.41]	-34.26	0.1	0.7518

Figure 7 - Taxa selection for MrBayes morphological phylogeny building

<i>Taxa</i>	<i>Classification</i>
1 <i>Ibalia.rufipes</i> , 2 <i>Andricus.quercusradicis</i> , 3 <i>Antistrophus.lygodesmiaepisum</i> , 4 <i>Asiocynips.lugubris</i> , 5 <i>Asiocynips.pannucea</i> , 6 <i>Aulacidea.phlomica</i> , 7 <i>Aulacidea.tragopogonis</i> , 8 <i>Aulacidea.verticillica</i> , 9 <i>Aylax.papaveris</i> , 10 <i>Barbotinia.oraniensis</i> , 11 <i>Biorhiza.pallida</i> , 12 <i>Cecconia.valerianellae</i> , 13 <i>Ceroptres.clavicornis</i> , 14 <i>Diastrophus.turgidus</i> , 15 <i>Diplolepis.rosae</i> , 16 <i>Eschatocerus.acaciae</i> , 17 <i>Euceroptres.montanus</i> , 18 <i>Gonaspis.potentillae</i> , 19 <i>Hedickiana.levantina</i> , 20 <i>Himalocynips.vigintilis</i> ,	1. Outgroup 2. Ingroup 3. Ingroup 4. Ingroup 5. Ingroup 6. Ingroup 7. Ingroup 8. Ingroup 9. Ingroup 10. Ingroup 11. Ingroup 12. Ingroup 13. Inquiline 14. Ingroup 15. Ingroup 16. Ingroup 17. Outgroup 18. Ingroup 19. Ingroup 20. Ingroup

21 <i>Iraella.luteipes</i> ,	21. Ingroup
22 <i>Isocolus.rogenhoferi</i> ,	22. Ingroup
23 <i>Liposthenes.glechomae</i> ,	23. Ingroup
24 <i>Liposthenes.kernerii</i> ,	24. Ingroup
25 <i>Neaylax.verbenaca</i> ,	25. Ingroup
26 <i>Neuroterus.numismalis</i> ,	26. Ingroup
27 <i>Panteliella.fedtschenkoi</i> ,	27. Ingroup
28 <i>Paramblynotus.zonatus</i> ,	28. Outgroup
29 <i>Parnips.nigripes</i> ,	29. Outgroup
30 <i>Pediaspis.aceris</i> ,	30. Ingroup
31 <i>Periclistus.brandtii</i> ,	31. Inquiline
32 <i>Phanacis.centaureae</i> ,	32. Ingroup
33 <i>Phanacis.hypochoeridis</i> ,	33. Ingroup
34 <i>Phanacis.phlomidis</i> ,	34. Ingroup
35 <i>Plagiotrochus.quercusilicis</i> ,	35. Ingroup
36 <i>Rhodus.oriundus</i> ,	36. Ingroup
37 <i>Synergus.crassicornis</i> ,	37. Inquiline
38 <i>Synophromorpha.rubi</i> ,	38. Inquiline
39 <i>Timaspis.phoenixopodos</i> ,	39. Ingroup
40 <i>Vetustia.investigata</i> ,	40. Ingroup
41 <i>Xestophanes.potentillae</i> ;	