Calligrapha:

Evolution of Behaviour, Metabolism and Reproduction in Unisexual and Bisexual Beetles

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Concepts & Objective

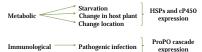
Calligrapha presents both diploid bisexuals and tetraploid unisexuals; unisexuals are parthenogenetic, they have arisen via asymmetric interspecific hybridisation and a consequent backcrossing with paternal species. Sexbiased genes related to behavioural, metabolic and reproductive functions will be identified and studied across a range of species and developmental stages. Including C. pantherina and C. scalaris, being of special concern due to their ecological roles as BCA and pest, respectively. The evolutionary trajectory of the genus will be drawn in relation to geographical distribution and host plant preference; examining the role of parthenogenesis and polyploidy. The genetic responses of these non-model organisms will be assessed in response to metabolic and immune stress.

Specific range

North American mountain ranges structure the genus in groups (Figure 1A): Pacific and Atlantic. Nonetheless, the main effect is due to the host plants they inhabit. Although, there is a case of geographical parthenogenesis in the area of the Great Lakes: it has the highest diversity and all unisexuals in North America; here, unisexuals sympatrically coexist with bisexuals.

Geographic and sympatric speciation occurs both on single and different host plants. Host shifts in phytophagous insects are often initiated by evolutionary changes in host preference, subsequently driving divergent selection and host adaptation.

Distinct responses to environmental stress appear as a result of acclimatization or adaptation of individuals to local conditions, which is determined by genetic differences and phenotypic plasticity.



Unisexual species

Sex-biased genes are largely responsible for intersexual variation, and are subject to the strong pressures associated to sexual selection, which derive in molecular evolution and speciation. These genes are found both in somatic and reproductive tissues, leading to anatomical, physiological and behavioural sexual dimorphisms. Reproductive strategy and ontological and development drive the evolution of sex-biased genes. Asexuality is rare within beetles: Calligrapha stands out with 7 parthenogenetic species. The genus counts with 80 species distributed from Alaska to Argentina; which share an intimate relationship with their host plants. Unisexual Calligrapha species have arisen via asymmetric interspecific hybridisation and a consequent backcrossing with paternal species (Figure 1B).

Tetraploidy

mtDNA diversity in probably all unisexual species derives from a yet unknown ancestral lineage. An ancestor probably hybridized during the Pliocene, leaving its descendants with polymorphic mtDNA; some of these female descendants went through a second wave of interspecific hybridisation during the Pleistocene to give rise to unisexual species (Figure 1C).

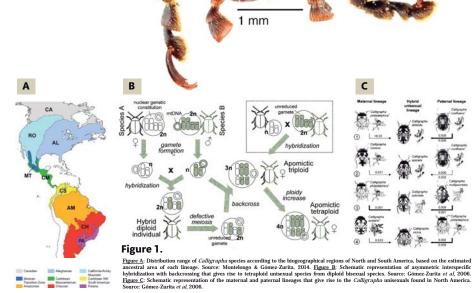
Calligrapha unisexuals are tetraploid with one set of chromosomes differing structurally from the other three; deriving from ancestral bisexual diploids. Each unisexual shares host plant species with its paternal lineage (p=0.0012); after inheriting the dominant allele for host plant preference from paternal species.

Specific Objectives

a. Identifying, sequencing and quantifying the expression of sexbiased genes with metabolic, reproductive and behavioural functions in various tissues and developmental stages of North American Calligrapha unisexuals, and their maternal and paternal lineages.

Species	Reproductive mode	Distribution	Host plant
C. alnicola	Unisexual	AL	Alnus
C. apicalis	Unisexual	AL	Alnus
C. confluens	Bisexual	AL	Alnus
C. multipunctata	Bisexual	AL, RO	Salix
C. pantherina	Bisexual	MT, CM	Sida
C. philadelphica	Bisexual	AL, RO	Cornus
C. rowena	Bisexual	AL	Cornus
C. scalaris	Unisexual or Bisexual	AL, RO, MT	Cornus
C. suturella	Unisexual	MT, RO	Salix
C. vicina	Unisexual	AL, RO	Cornus

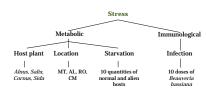
- b. Determine if host plant preference is a trait controlled by male-
- c. Study the relationship between genetic evolution and ecological factors, relating it to geographical distribution and host plant preference.
- d. Study how potentially stressful situations, affects gene expression of key behavioural, metabolic and reproductive genes.
- e. Relate Calligrapha pantherina's genetic background to its use as a BCA against Sida in Australia and the potential threat it poses to native plants in Australia and New Caledonia.
- f. Study the genetic factors allowing Calligrapha scalaris to act as a serious pest of Ulmus Americana.
- g. Analyse evolution of sex-biased genes in unisexuals and bisexuals throughout their development; comparing unisexuals with their maternal and paternal lineages, and other species.



Methodological Approach

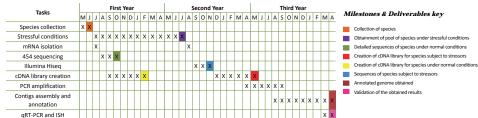
- Collection of specimens -larvae, pupae and adults in their natural habitats during an entomological campaign.
- 2. Pool of species under stressful conditions
- Isolation of mRNA from cerebral, gonadal, and fat body tissues from larvae, pupae and adults of each species
- 4. 454 Sequencing of each specimen under natural conditions.
- Illumina Hiseq 2000 of the main genes from each tissue for the first reared generation under stressful conditions.
- 6. Create cDNA libraries from tissue transcriptomes.
- 7. Amplify sequences using PCR.
- Assembly and functional annotation of contigs obtained by using Geneious software: use BLASTs from NCBI, Gene Ontology, and GenBank tools.
- 9. qRT-PCR and ISH to validate the DEGs obtained from the RNA-sequencing.

Outline of experimental stress tests

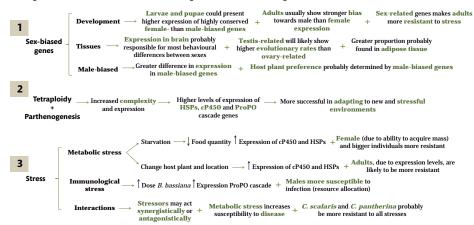


Stressors will be evaluated separately and combined

Work Plan



Expected Results & Specific Output



Selected References

Gómez-Zurita, J., Funk, D.J., Vogler, A.P. 2006. The Evolution of Unisexuality in Calligrapha Leaf Beetles: Molecular and Ecological Insights on Multiple Origins Via Interspecific Hybridization. Evolution 60 (2): 328-347. Montelongo, T., Gómez-Zurita, J. 2014. Multilocus molecular systematics and evolution in time and space of Calligrapha (Coleoptera: Chrysomelidae, Chrysomelinae). Zoologica Scripta 43 (6): 605-628. Montelongo, T., Gómez-Zurita, J. 2015. Nonrandom Patterns of Genetic Admixture Expose the Complex Historical Hybrid Origin of Unisexual Leaf Beetle Species in the Genus Calligrapha Endersical Manerican Maturalist 185 (1): 113-134. Haerty, W., Jagadeeshana, S., Kutalthinal, B.J., Wong, A., Ram, K.R., Sirot, L.K., Levesque, L., Artieri, C.G., Wolfner, M.F., Civetta, A., Singh, R.S. 2007. Evolution in the Fast Lane: Rapidly Evolving Sex-Related Genes in Drosophila. Genetics 177: 1321-1335.