



Cenicafé
Centro Nacional de Investigaciones de Café



De los genes a los granos: el papel de la biología molecular en la salud humana, la agricultura y la historia del café

Marco Aurelio Cristancho – Disciplina de Fitopatología

Temas

- Historia de las Tecnologías Moleculares
 - Humanos
 - Animales
 - Plantas
- Aplicaciones en la Agricultura
 - Café
- Presente y Futuro de las tecnologías moleculares en café

Historia: 1953



Watson y Crick

equipment, and to Dr. G. E. B. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

- *Young, F. B., Gerratt, H., and Jervis, W., *Phil. Mag.*, **46**, 149 (1950).
- *Lengmuir, H., *J. Chem. Phys.*, **16**, 1059 (1948).
- *Van Arman, W. S., *Wood's Hole Papers in Phys. Geol. Meteor. Sci.*, **11**, 25 (1952).
- *Frisson, V. W., *Arch. Biochem. Biophys.*, **2**(11) (1953).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxy-ribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's² model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There



This figure is partly representative of the two phosphate-sugar chains, and the horizontal rungs are the pairs of bases holding the chains together. The vertical line marks the fibre axis.

is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on those assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{3,4} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

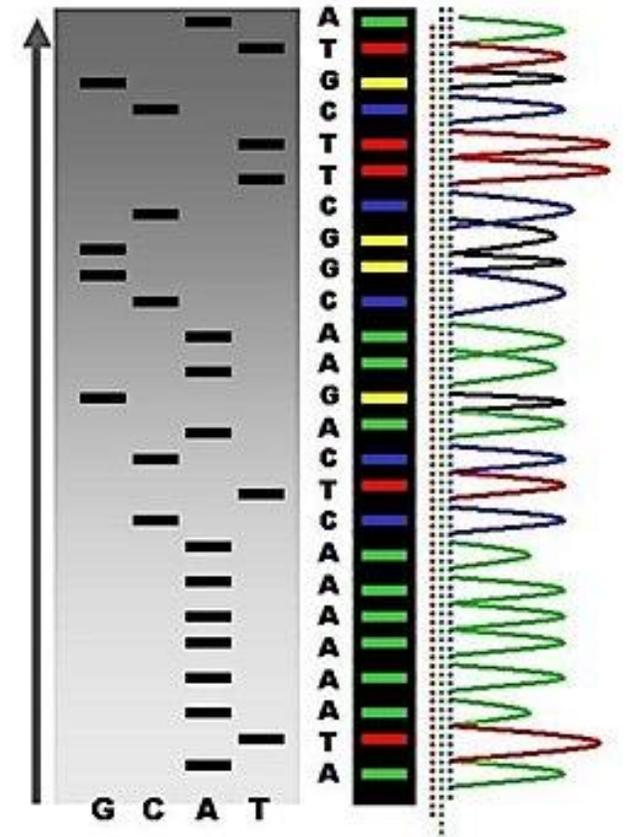
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

Historia: 1977

Secuenciación del ADN

Método Maxam–Gilbert

Método Sanger



PCR - 1986



Kary Mullis

Kary B. Mullis

Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction

K. MULLIS, F. FALOONA, S. SCHARF, R. SAIKI, G. HORN, AND H. ERLICH
Cetus Corporation, Department of Human Genetics, Emeryville, California 94608

The discovery of specific restriction endonucleases (Smith and Wilcox 1970) made possible the isolation of discrete molecular fragments of naturally occurring DNA for the first time. This capability was crucial to the development of molecular cloning (Cohen et al. 1973); and the combination of molecular cloning and endonuclease restriction allowed the synthesis and isolation of any naturally occurring DNA sequence that could be cloned into a useful vector and, on the basis of flanking restriction sites, excised from it. The availability of a large variety of restriction enzymes (Roberts 1985) has significantly extended the utility of these methods.

The de novo organic synthesis of oligonucleotides and the development of methods for their assembly into long double-stranded DNA molecules (Davies and Gassen 1983) have removed, at least theoretically, the minor limitations imposed by the availability of natural sequences with fortuitously unique flanking restriction sites. However, de novo synthesis, even with automated equipment, is not easy; it is often fraught with peril due to the inevitable indelicacy of chemical reagents (Urdea et al. 1985; Watt et al. 1985; Mullenbach et al. 1986), and it is not capable of producing, intentionally, a sequence that is not yet fully known.

We have been exploring an alternative method for the synthesis of specific DNA sequences (Fig. 1). It involves the reciprocal interaction of two oligonucleotides and the DNA polymerase extension products whose synthesis they prime, when they are hybridized to different strands of a DNA template in a relative orientation such that their extension products overlap. The method consists of repetitive cycles of denaturation, hybridization, and polymerase extension and seems not a little boring until the realization occurs that this procedure is catalyzing a doubling with each cycle in the amount of the fragment defined by the positions of the 5' ends of the two primers on the template DNA, that this fragment is therefore increasing in concentration exponentially, and that the process can be continued for many cycles and is inherently very specific.

The original template DNA molecule could have been a relatively small amount of the sequence to be synthesized (in a pure form and as a discrete molecule) or it could have been the same sequence embedded in a much larger molecule in a complex mixture as in the case of a fragment of a single-copy gene in whole human DNA. It could also have been a single-stranded

DNA molecule or, with a minor modification in the technique, it could have been an RNA molecule. In any case, the product of the reaction will be a discrete double-stranded DNA molecule with termini corresponding to the 5' ends of the oligonucleotides employed.

We have called this process polymerase chain reaction or (inevitably) PCR. Several embodiments have been devised that enable one not only to extract a specific sequence from a complex template and amplify it, but also to increase the inherent specificity of this process by using nested primer sets, or to append sequence information to one or both ends of the sequence as it is being amplified, or to construct a sequence entirely from synthetic fragments.

MATERIALS AND METHODS

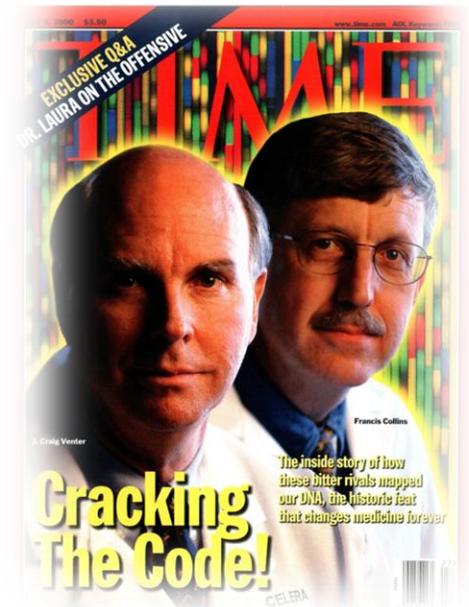
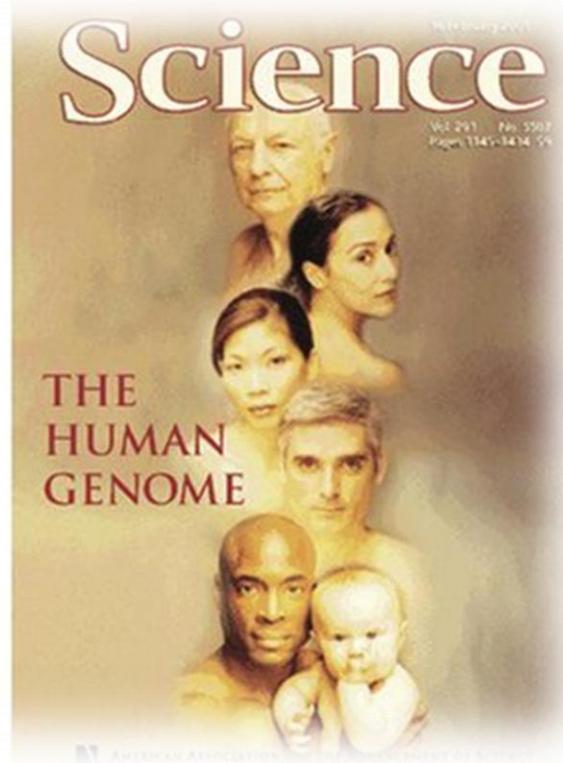
PCR amplification from genomic DNA. Human DNA (1 μ g) was dissolved in 100 μ l of a polymerase buffer containing 50 mM NaCl, 10 mM Tris-Cl (pH 7.6), and 10 mM MgCl₂. The reaction mixture was adjusted to 1.5 mM in each of the four deoxynucleoside triphosphates and 1 μ M in each of two oligonucleotide primers. A single cycle of the polymerase chain reaction was performed by heating the reaction to 95°C for 2 minutes, cooling to 30°C for 2 minutes, and adding 1 unit of the Klenow fragment of *Escherichia coli* DNA polymerase I in 2 μ l of the buffer described above containing about 0.1 μ l of glycerol (Klenow was obtained from U.S. Biochemicals in a 50% glycerol solution containing 5 U/ μ l). The extension reaction was allowed to proceed for 2 minutes at 30°C. The cycle was terminated and a new cycle was initiated by returning the reaction to 95°C for 2 minutes. In the amplifications of human DNA reported here, the number of cycles performed ranged from 20 to 27.

Genotype analysis of PCR-amplified genomic DNA using ASO probes. DNA (1 μ g) from various cell lines was subjected to 25 cycles of PCR amplification. Aliquots representing one thirtieth of the amplification mixture (33 ng of initial DNA) were made 0.4 M in NaOH, 25 mM in EDTA in a volume of 200 μ l and applied to a Genatran-45 nylon filter with a Bio-Dot spotting apparatus. Three replicate filters were prepared. ASO probes (Table I) were 5'-phosphorylated with [γ -³²P]ATP and polynucleotide kinase and purified by spin dialysis. The specific activities of the probes were between 3.5 and 4.5 μ Ci/pmol. Each filter

From: <https://evilleeye.com/in-the-neighborhood/nobel-prize-winning-emeryville-scientist-kary-mullis-passes-at-74/>

Historia: 2003

Proyecto Genoma Humano



El Genoma es Muy Complejo: Genes Individuales

- **Pico de viuda** (línea de implantación del cabello en forma de V): alelo dominante (W).



El Genoma es Muy Complejo: Genes Individuales

- Capacidad para enrollar la lengua: depende en general de un solo gen



Test Genéticos: 2013



- BRCA1 y BRCA2, aumentan significativamente el riesgo de cáncer de mama, ovario, próstata y otros
- Su madre, tía y abuela fallecieron de cáncer
- Otras consideraciones del estilo de vida (Fumadores – Alcohol – Dieta)
- BRCA2 (+):
 - 87 % de riesgo de cáncer de mama a lo largo de la vida (12 % en la población general).
 - 50 % de riesgo de cáncer de ovario (frente a aproximadamente el 1,3 %).
- Cirugía preventiva:
 - Mastectomía doble: Redujo su riesgo de cáncer de mama a menos del 5 %.
 - Ooforectomía: Cirugía de ovarios y trompas de Falopio.

*Medicina de Precisión

Aplicaciones:

Medicina Forense

Antropología

Neanderthal y homo sapiens

Estudios de paternidad

Biología Evolutiva – Mamut

Virología – Covid19



O.J. Simpson

Aplicaciones:

Medicina Forense

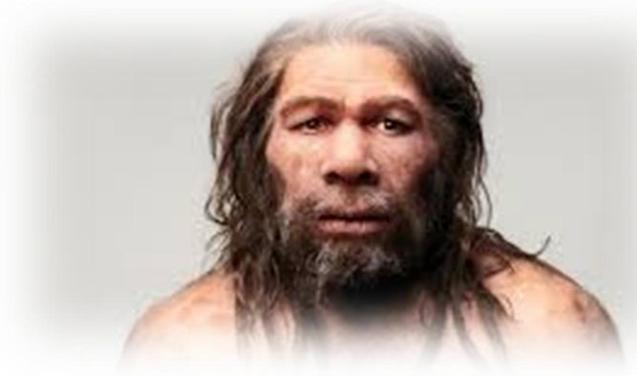
Antropología

Estudios de paternidad

Biología Evolutiva – Mamut

Virología – Covid19

Neanderthal



Homo sapiens



Aplicaciones:

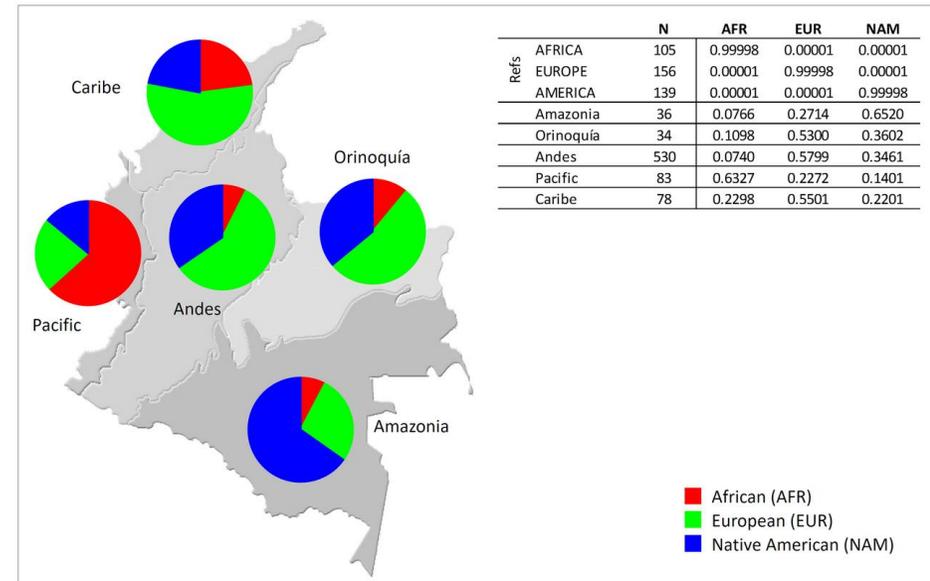
Medicina Forense

Antropología

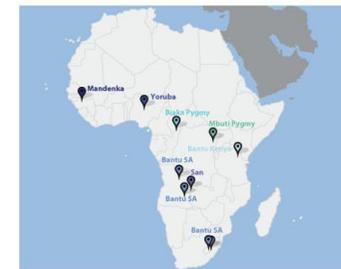
Estudios de paternidad

Biología Evolutiva – Mamut

Virología – Covid19



Sub-continental origins of an Afro-Colombian genome



Geographic Origin	Coordinates	Tribe	No. LCLs	No. Males
Central African Republic	4N, 17E	Biaka Pygmy relatives	36	33
Democratic Republic of Congo	1N, 29E	Mbuti Pygmy relatives	15	13
Senegal	12N, 12W	Mandenka relatives	24	16
Nigeria	6-10N, 2-8E	Yoruba relatives	25	13
Namibia	21S, 20E	San relatives	7	7
Kenya	3S, 37E	Bantu NE relatives	12	11
Bantu Speakers S. Africa			8	8
S. Africa Bantu S.E.	29S, 30E	Bantu S.E. Pedi	1	1
S. Africa Bantu S.E.	29S, 29E	Bantu S.E. Sotho	1	1
S. Africa Bantu S.E.	28S, 24E	Bantu S.E. Tswana	2	2
S. Africa Bantu S.E.	28S, 31E	Bantu S.E. Zulu	1	1
S. Africa Bantu S.W.	22S, 19E	Bantu S.W. Herero	2	2
S. Africa Bantu S.W.	19S, 18E	Bantu S.W. Ovambo	1	1
SUBSAHARAN AFRICA			127	109

Source: HGDP-CEPH Project - <https://www.cephb.fr/en/hado/table.php>

Ossa, H., Aquino, J., Pereira, R., Ibarra, A., Ossa, R. H., Pérez, L. A., ... & Gusmão, L. (2016). Outlining the ancestry landscape of **Colombian admixed populations**. PloS one, 11(10), e0164414.

Aplicaciones:

Medicina Forense

Antropología

Estudios de paternidad

Biología Evolutiva – Mamut

Virología – Covid19



I am your father!

Aplicaciones:

Medicina Forense

Antropología

Estudios de paternidad

Biología Evolutiva

Virología – Covid19



Estudio de Organismos extintos

Aplicaciones:

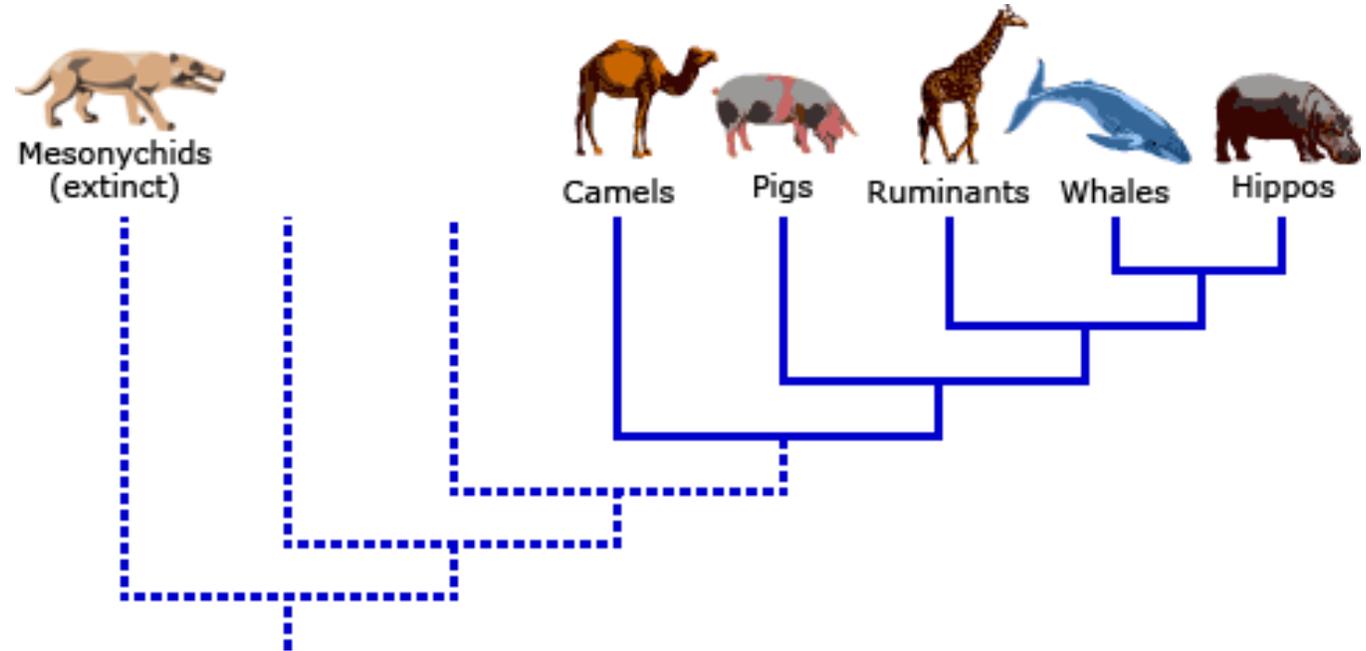
Medicina Forense

Antropología

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Virología – Covid19



<https://evolution.berkeley.edu/phylogenetic-systematics/using-trees/using-trees-to-make-predictions-about-fossils-the-whales-ankle/>

Aplicaciones:

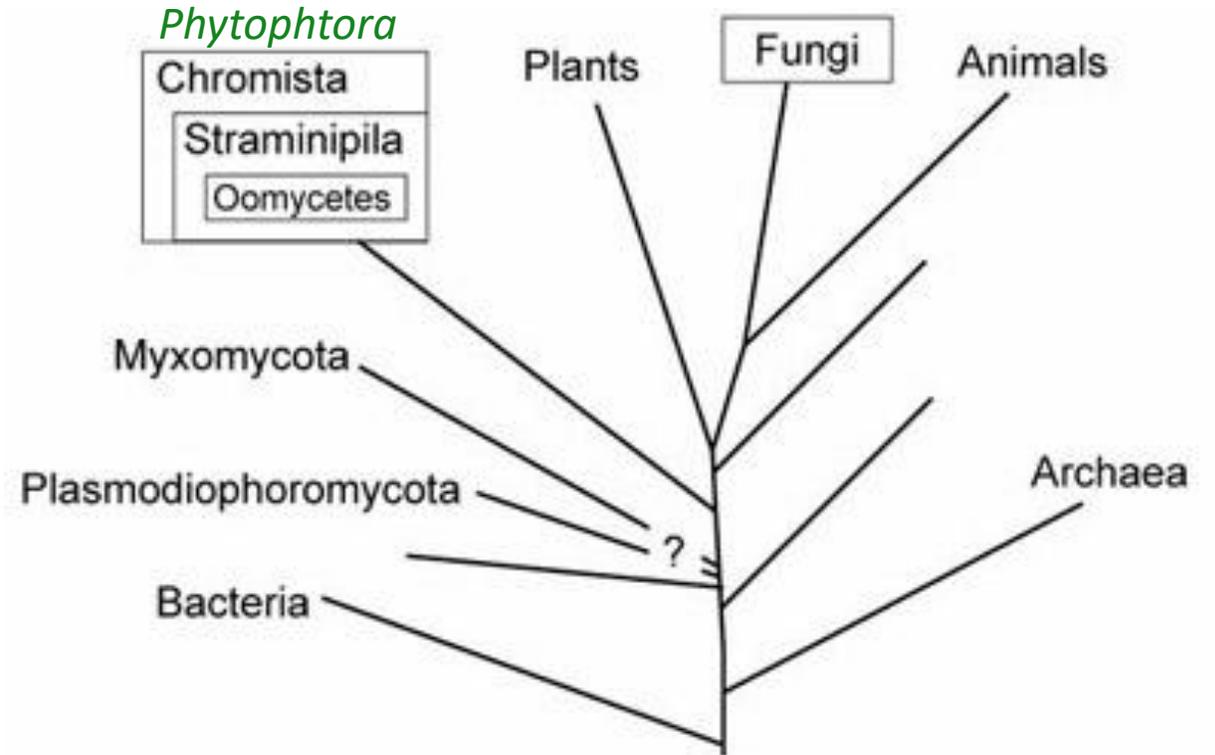
Medicina Forense

Antropología

Estudios de paternidad

Biología Evolutiva

Virología – Covid19



<https://www.apsnet.org/edcenter/Pages/OomycetesLab.aspx>

Aplicaciones:

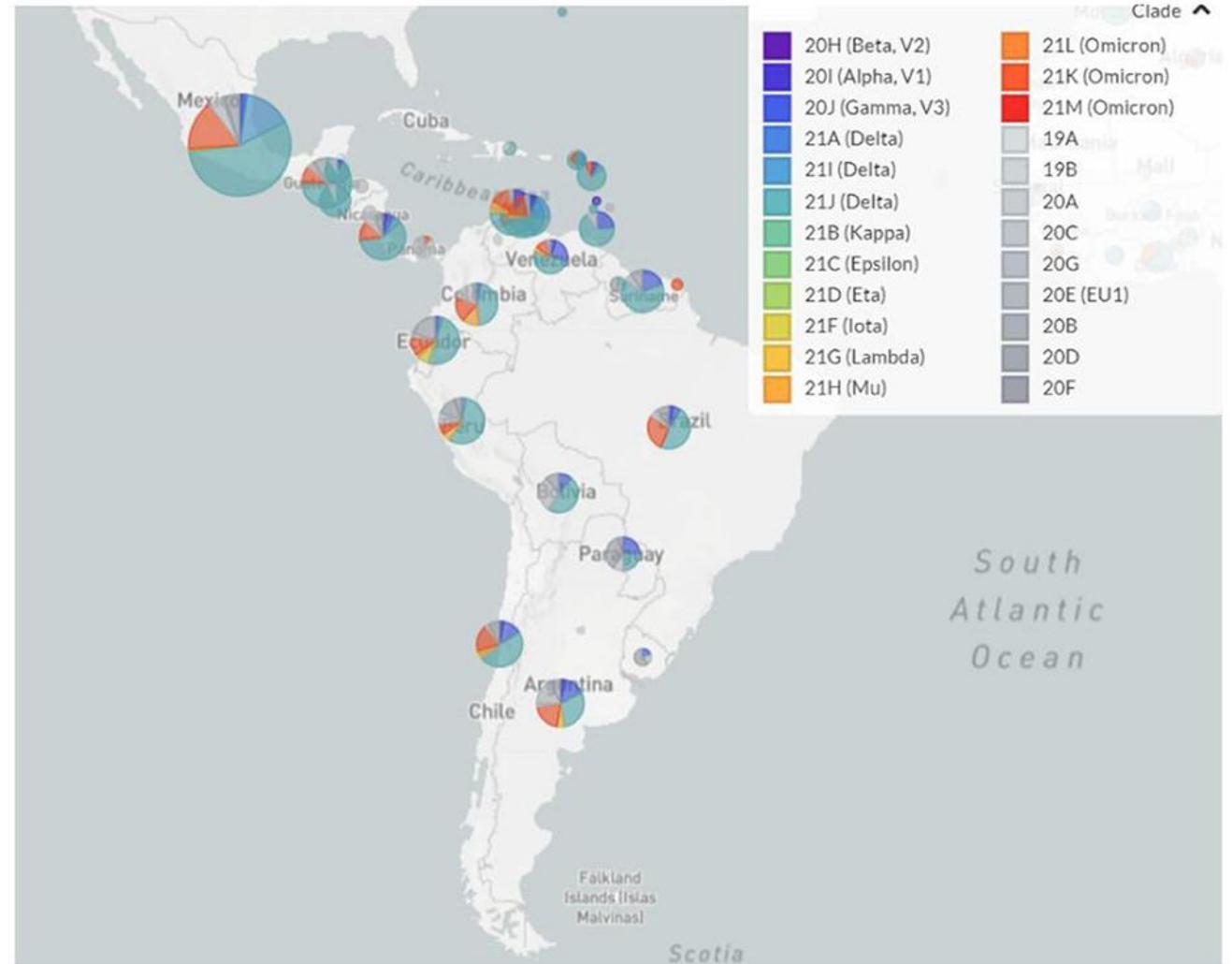
Medicina Forense

Antropología

Estudios de paternidad

Biología Evolutiva

Virología – Covid19



Molina-Mora, J. A., Reales-González, J., Camacho, E., Duarte-Martínez, F., Tsukayama, P., Soto-Garita, C., **Cristancho**, M. & Herrera-Estrella, A. (2023). Overview of the **SARS-CoV-2 genotypes circulating in Latin America** during 2021. *Frontiers in Public Health*, 11, 1095202.

Efecto de la cafeína

- El gen **CYP1A2** codifica la enzima hepática responsable de metabolizar la cafeína.

- **Metabolizadores rápidos (~40-50% de las personas)**

- Poseen el genotipo **CYP1A2*1A/*1A** (enzima de alta actividad)

- **Metabolizadores lentos (~50-60% de las personas)**

- Son portadores de al menos un alelo **CYP1A2*1F** (*1A/*1F o *1F/*1F)

Biología Molecular en la Agricultura:

Primera planta genéticamente modificada (1983)

El tomate Flavr Savr, diseñado para una maduración retardada



Comercialización de cultivos transgénicos (1996)

Soja Roundup Ready de Monsanto, tolera herbicidas

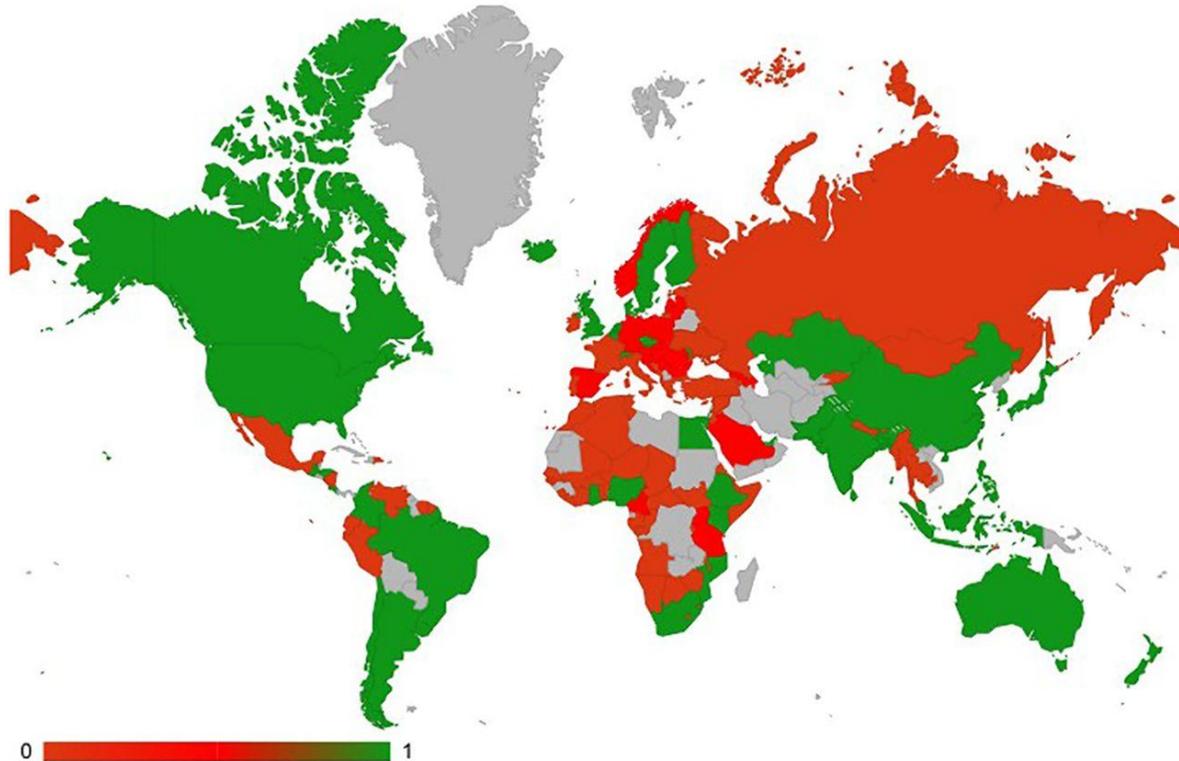


Introducción de cultivos Bt (1996)

Resistencia a insectos



Adopción global de cultivos transgénicos (década de 2000)



	Cotton	Maize	Soybean	Sugarcane
Argentina		NA (Pvt)	NA (Pvt)	
Brazil	NA (Pvt)	NA (Pvt)	NA (Pvt)	NA (Pub)
Canada		IR (Pvt)	NR (Pvt)	
Colombia	NA (Pvt)	NA (Pvt)	NA (Pvt)	
Costa Rica	NA (Pub)			
Paraguay		NA (Pvt)	NA (Pvt)	

- Biotic Stress Resistance
- Herbicide Tolerance
- Industrial Application
- Yield Improvement
- Nutritional Improvement
- Abiotic Stress Resistance
- Research Purposes

Ricroch, A., Desachy, L. D., Penfornis, M., Akin, M., Kondić-Špika, A., Kuntz, M., & Miladinović, D. (2024). Worldwide study on field trials of **biotechnological crops**: new promises but old policy hurdles. *Frontiers in Plant Science*, 15, 1452767.

Café: Genes Individuales

Características morfológicas y de desarrollo

- Enanismo: **Caturra**
- Forma de la hoja: Gen Lm1 (mutación **lanceolada** en *C. arabica*)
- Color de la semilla (grano): Gen Y (grano amarillo vs. **verde** en *C. arabica*)

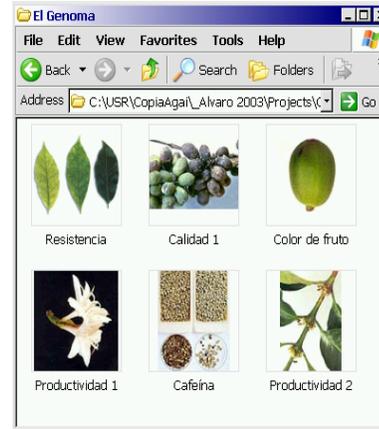


Tolerancia a estrés abiótico

- Tolerancia a la **sequía**: CcDREB1D (gen del factor de transcripción en Robusta)

Proyecto del Genoma del Café: 2003 - 2014

“Iniciativa para el estudio del genoma del café, de la broca y de su agente biocontrolador, el hongo *Beauveria bassiana*”



30,000 Genes



Dr. Gabriel Cadena

José Ricardo Acuña, Pablo Benavides, Marco Aurelio Cristancho, Alvaro León Gaitán, Carmenza Góngora, Juan Carlos Herrera, Diana Molina, María Del Pilar Moncada, Húver Posada *
Mención de Honor - Ciencias 2008

Proyecto Genoma del Café

**Grupo de Bioinformática de
CENICAFÉ**

**Primer grupo del área en
Colombia**



Proyecto Genoma

Genomas Café

C. arabica var. Caturra

C. arabica Di-Haploide

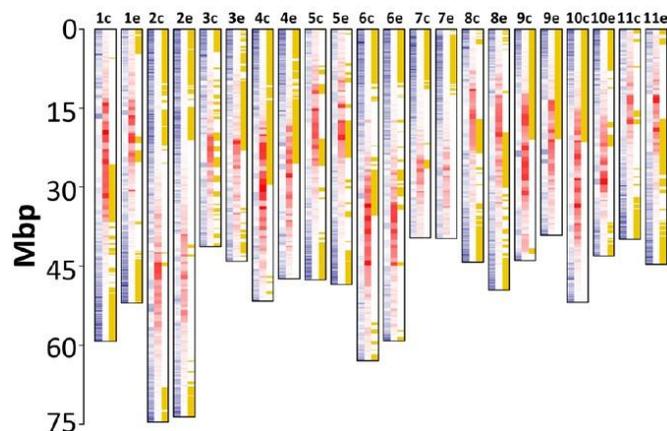
C. canephora

C. eugenioides

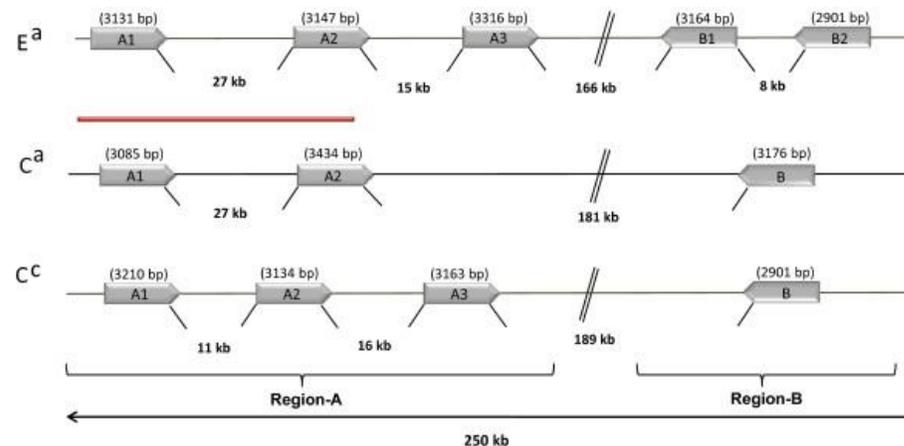
C. liberica

Híbrido de Timor

Etiopia: 90 accessiones



Genes de Resistencia



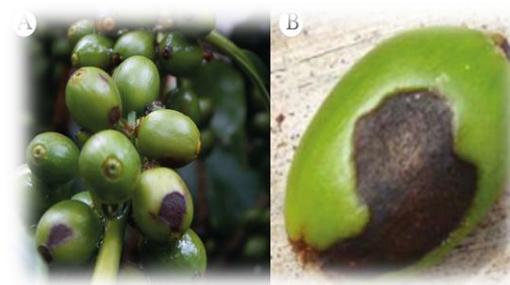
SH3 Gene

KASP Markers Mapping

SH6
SH7
SH8
SH9



Caracterización de *C. arabica* por resistencia al CBD



eISSN: 1984-3909
Coffee Science, e192230, 2024

Doi: <https://doi.org/10.25186/v19i.2230>

Characterization in populations of *Coffea arabica* L. for resistance to CBD using molecular markers

Luisa F. López-Monsalve¹ , Julio Quiroga-Cardona¹ , Natalia Arango López² ,
Carlos A. Ramírez-Cardona¹ , Claudia P. Flórez-Ramos¹ 

¹Plant Breeding, National Coffee Research Center, Cenicafé. Chinchiná, Caldas, Colombia

²Plant Science Program, Biological and Environmental Science and Engineering Division/BESE, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

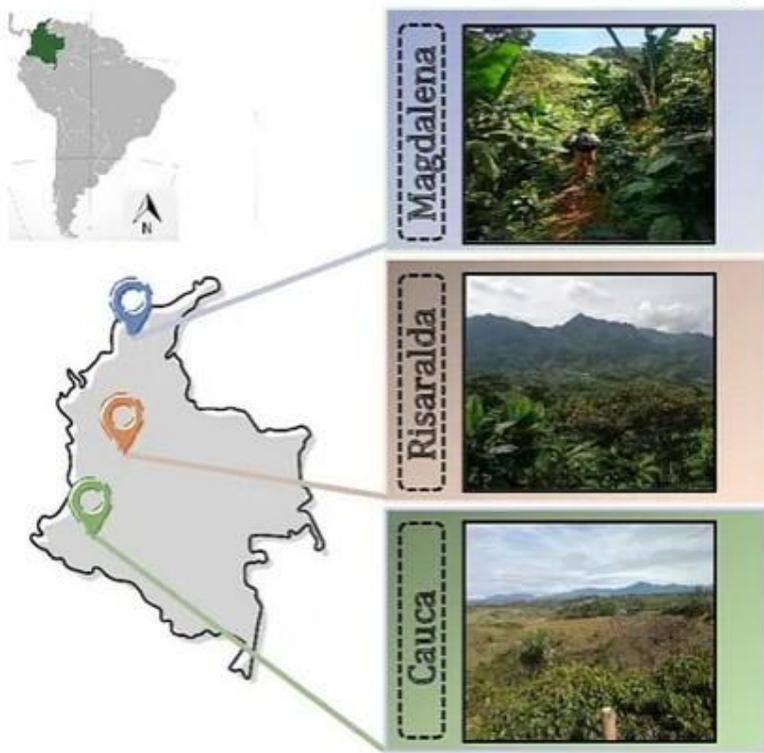
Contact authors: luisa.lopez@cafedecolombia.com; julio.quiroga@cafedecolombia.com; natalia.arangolopez@kaust.edu.sa; carlos.ramirez@cafedecolombia.com;

claudia.florez@cafedecolombia.com

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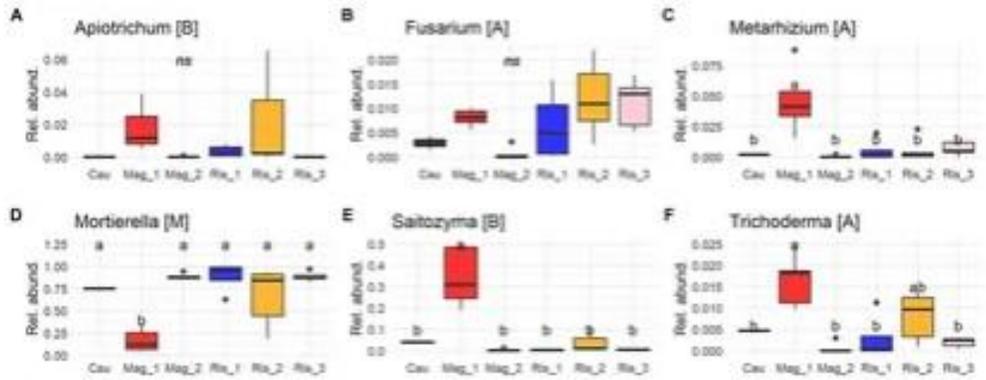
Microbiología Suelos del Café

Figure 1



Metagenómica

Figure 4

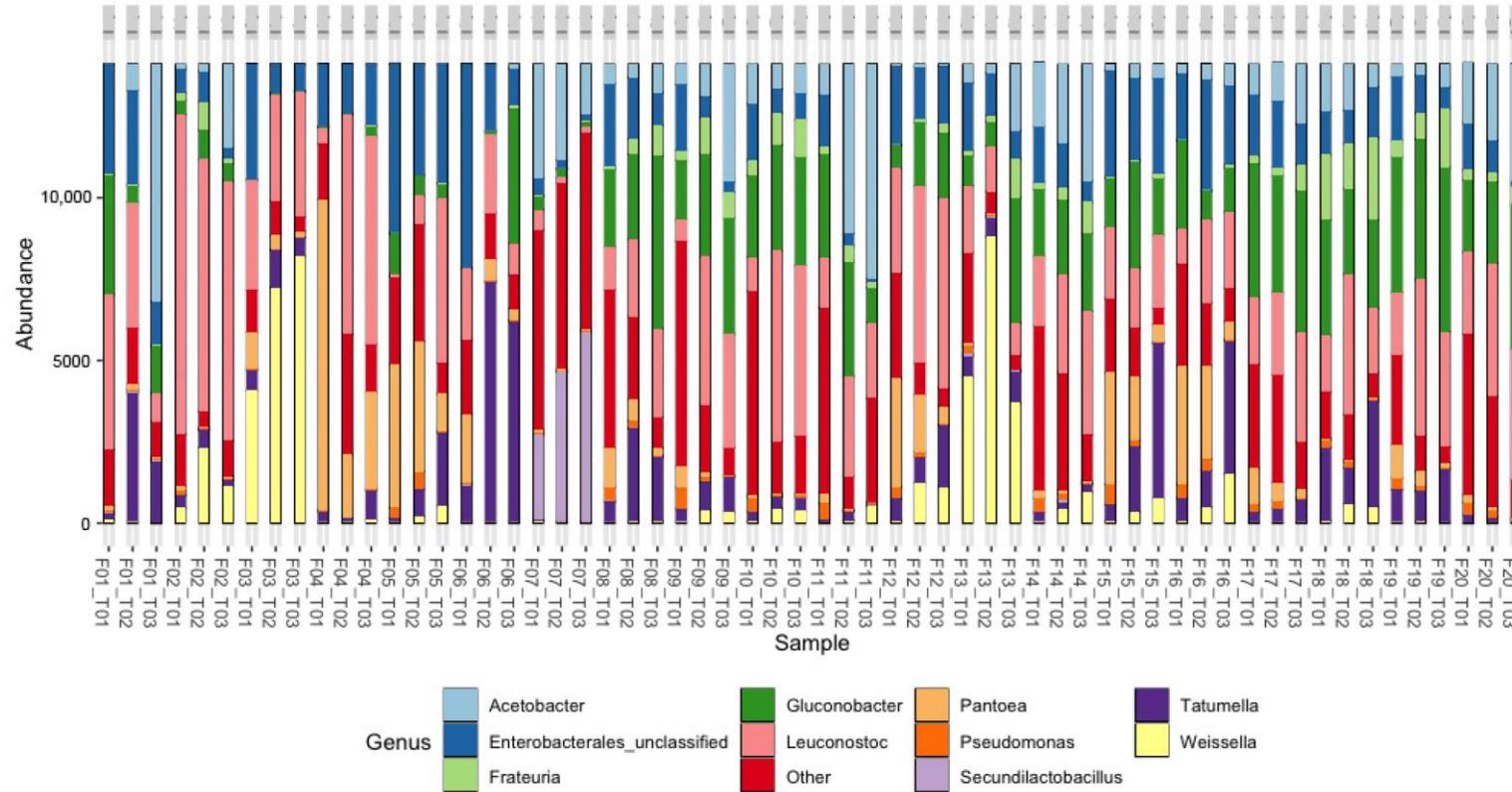


Ochoa-Henriquez, V. H., Faggioli, V., Gómez-Godínez, L. J., Rivarola, M., & **Cristancho, M.** (2024). **Colombian coffee** (*Coffea arabica* L.) plantations: a taxonomic and functional survey of 1345383 soil fungi. *Frontiers in Sustainable Food Systems*, 8, .

Fermentación del Café

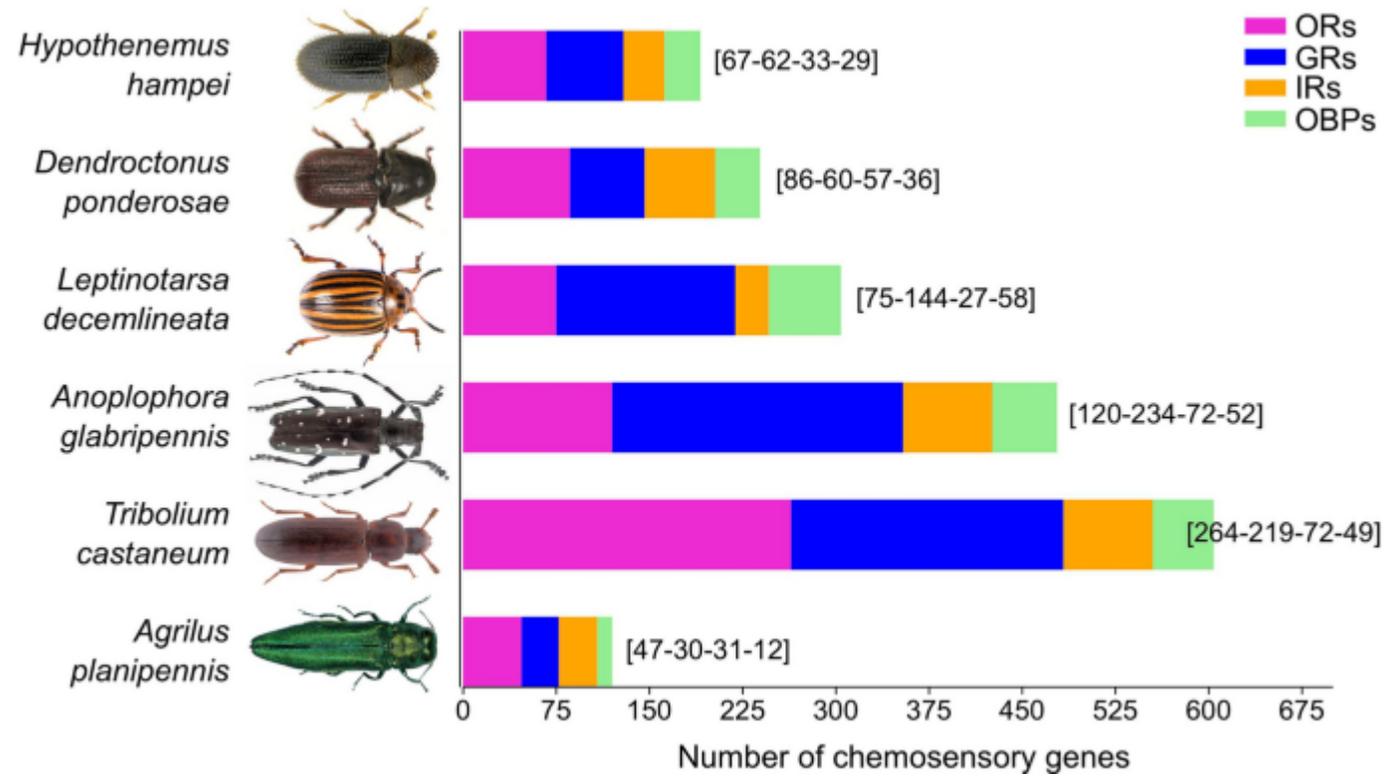


Metagenómica



Góngora, C. E., Holguín-Sterling, L., Pedraza-Claros, B., Pérez-Salinas, R., Ortiz, A., & Navarro-Escalante, L. (2024). Metataxonomic identification of microorganisms during the coffee fermentation process in Colombian farms (Cesar department). *Foods*, 13(6), 839.

Genómica de la Broca



Navarro-Escalante, L., Hernandez-Hernandez, E. M., Nuñez, J., Acevedo, F. E., Berrio, A., Constantino, L. M., ... & Benavides, P. (2021). A coffee berry borer (***Hypothenemus hampei***) genome assembly reveals a reduced chemosensory receptor gene repertoire and male-specific genome sequences. *Scientific reports*, 11(1), 4900.

Genoma de *Hemileia vastatrix*

ORIGINAL RESEARCH article

Front. Plant Sci., 30 October 2014

Sec. Plant Pathogen Interactions

Volume 5 - 2014 | <https://doi.org/10.3389/fpls.2014.00594>

This article is part of the Research Topic

Genomics research on non-model plant pathogens: delivering novel insights into rust fungus biology.

[View all 15 articles >](#)

Annotation of a hybrid partial genome of the coffee rust (*Hemileia vastatrix*) contributes to the gene repertoire catalog of the Pucciniales



¹ Plant Pathology, National Center for Coffee Research – CENICAFÉ, Chinchiná, Colombia

² Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia

³ Institute for Cereal Crops Improvement, Tel Aviv University, Tel Aviv, Israel

Coffee leaf rust caused by the fungus *Hemileia vastatrix* is the most damaging disease to coffee worldwide. The pathogen has recently appeared in multiple outbreaks in coffee producing countries resulting in significant yield losses and increases in costs related to its control. New races/isolates are constantly emerging as evidenced by the presence of the fungus in plants that were previously resistant. Genomic studies are opening new avenues for the study of the evolution of pathogens, the

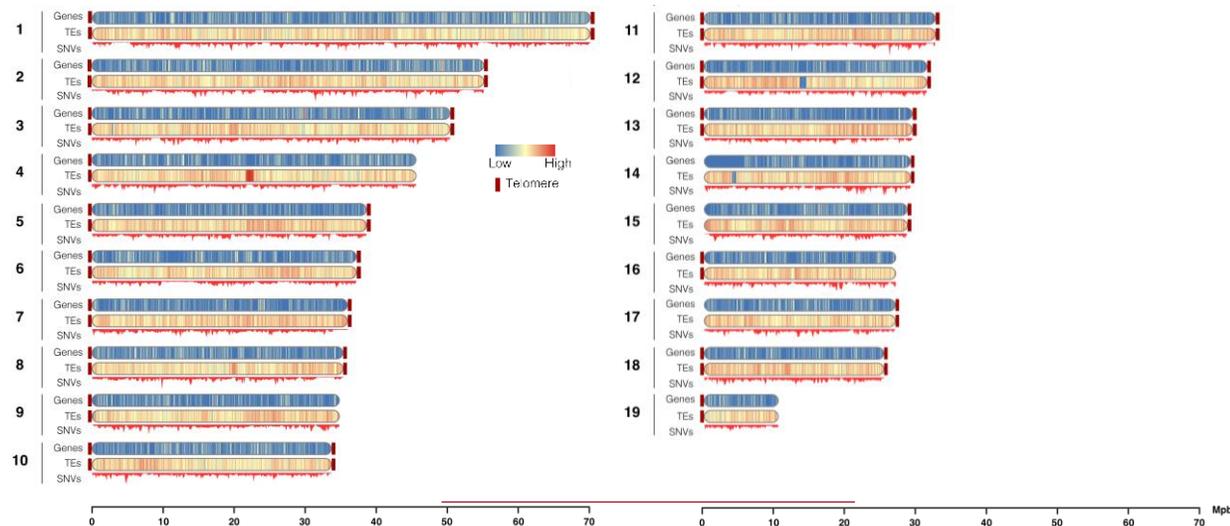
Genoma gigante

750 – 790 Mb!!

Genoma de *Hemileia vastatrix* raza I

Assembly

Total Length (bp)	773,428,567
N50	35,486,499
L50	9
N90	55
L90	26
Largest scaffold	71,415,227
Number of scaffolds	914
Number of gaps	29
Complete BUSCOs*	96.08%
GC (%)	33.8



13.045 genes
81,43% TEs

Angel, Marín & Maldonado, 2023

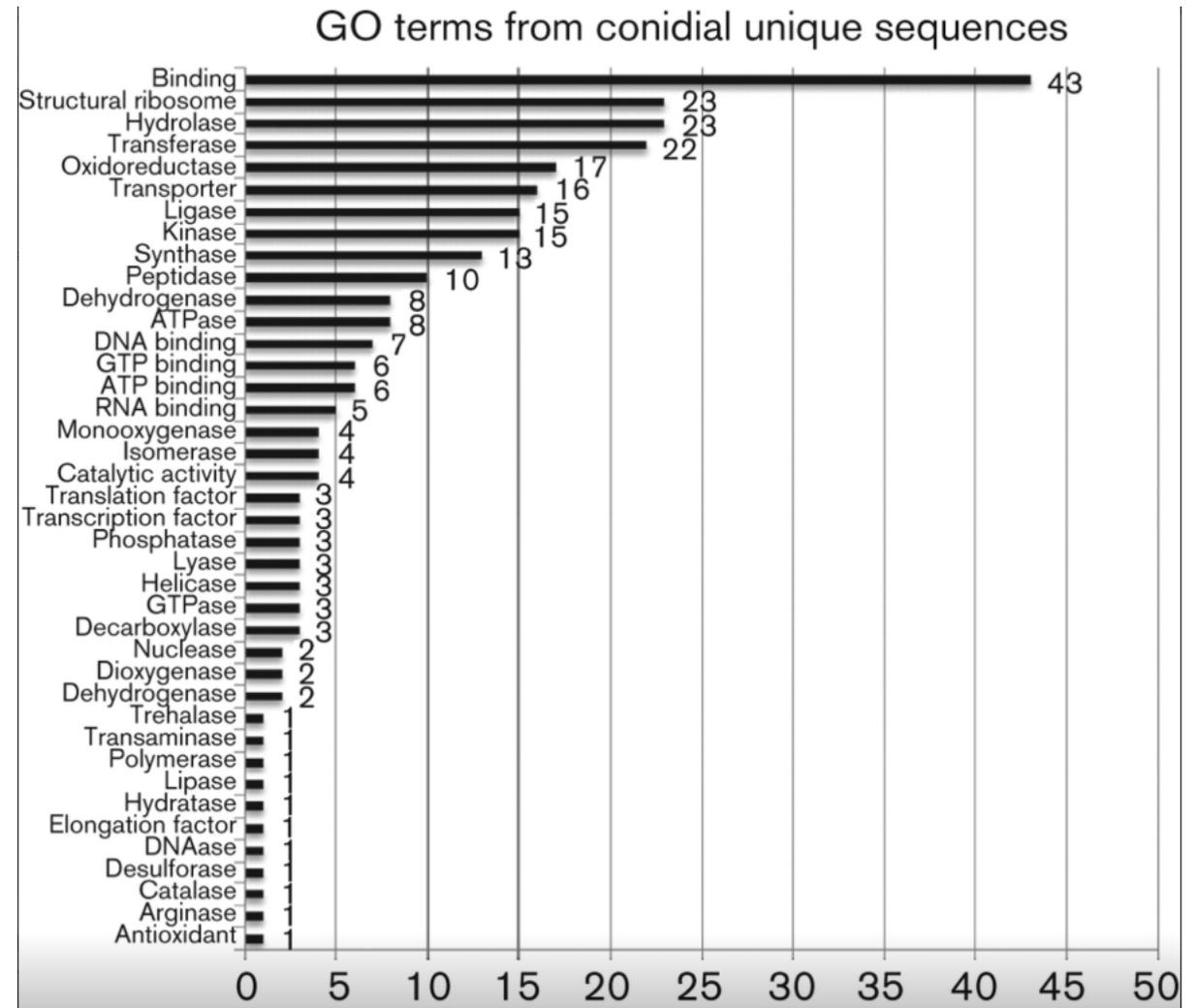


Microbiology
Resource Announcements

Cenicafé
Centro Nacional de Investigaciones de Café

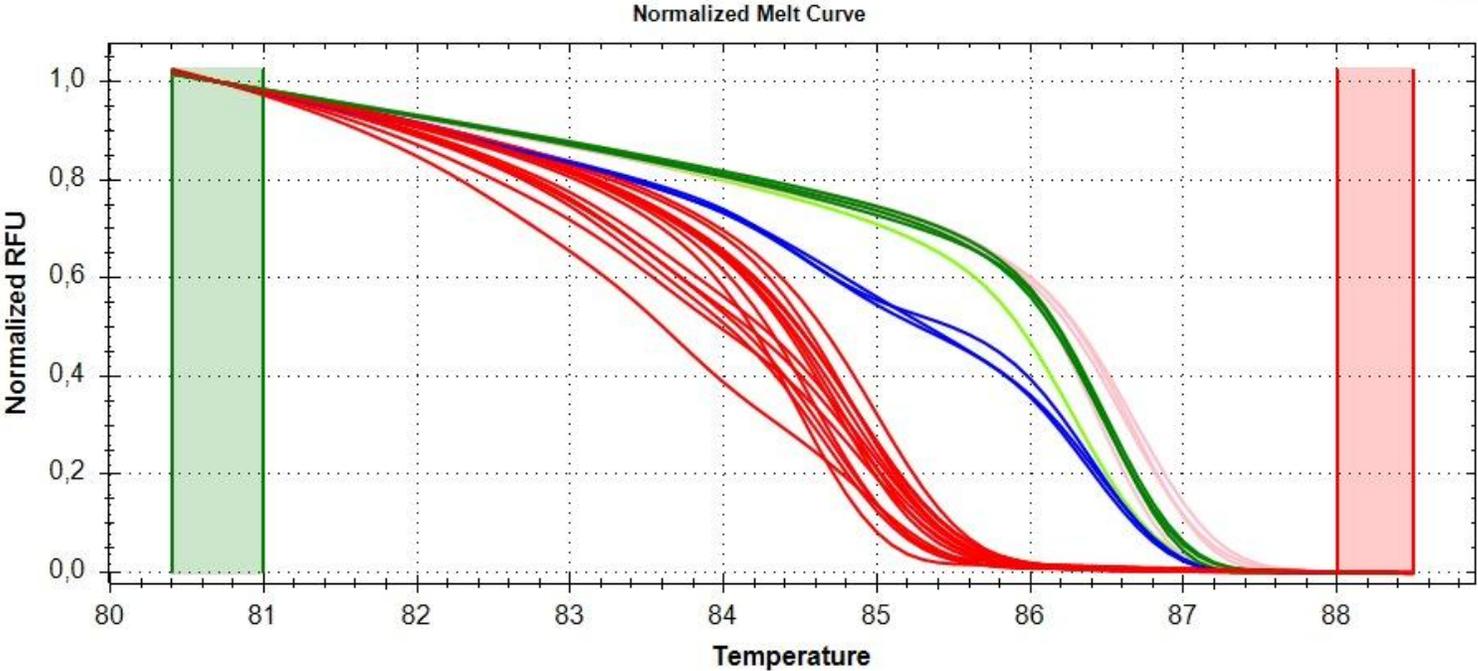
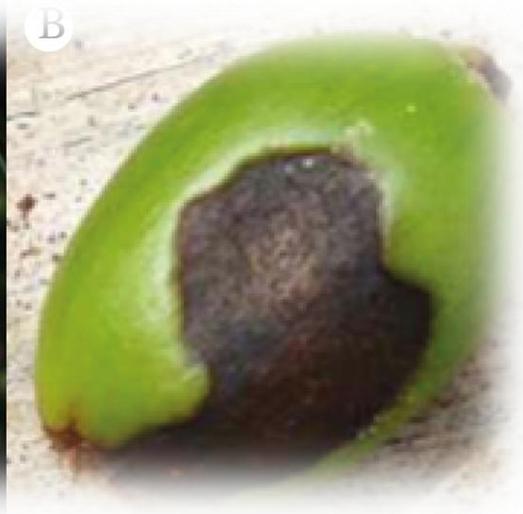


Genómica de *Beauveria bassiana*



Mantilla, J. G., Galeano, N. F., Gaitan, A. L., Cristancho, M. A., Keyhani, N. O., & Gongora, C. E. (2012). Transcriptome analysis of the entomopathogenic fungus *Beauveria bassiana* grown on cuticular extracts of the coffee berry borer (*Hypothenemus hampei*). *Microbiology*, 158(7), 1826-1842.

CBD – Diagnóstico Molecular



Presente y Futuro!

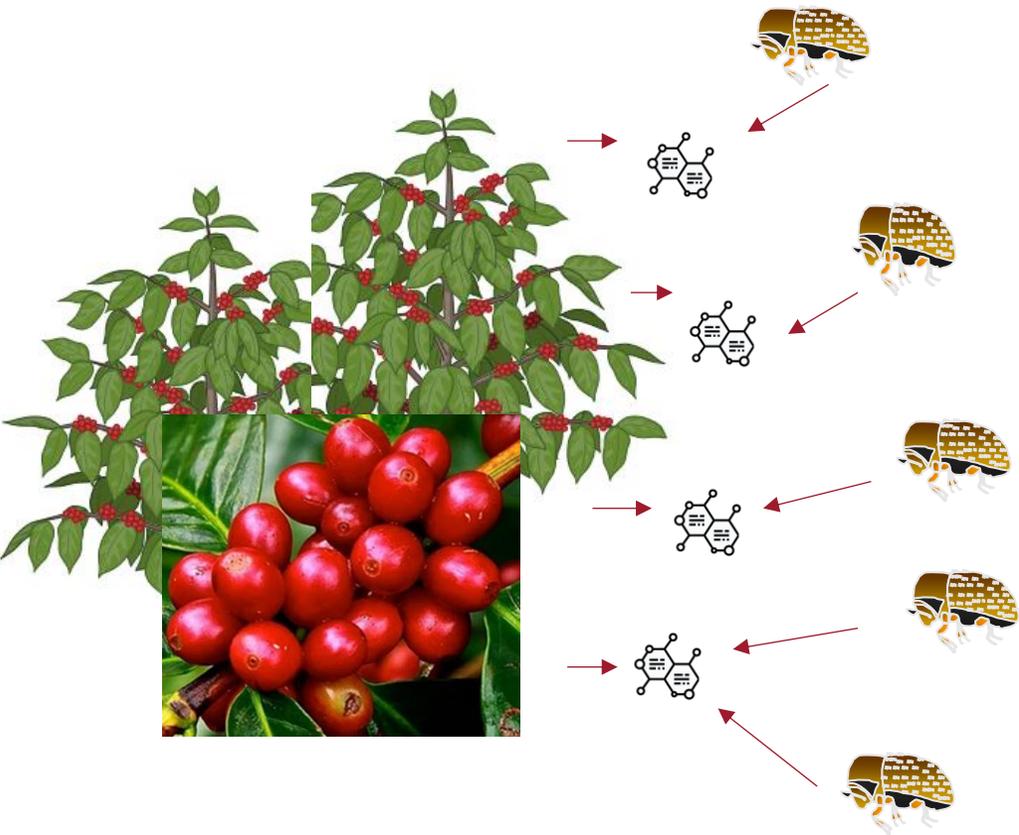
Edición de Genes (CRISPR-Cas9)



Jennifer Doudna and Emmanuelle Charpentier
Premio Nobel - 2020

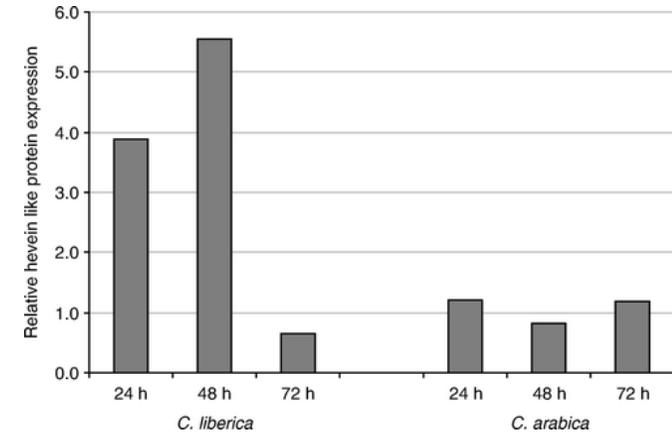
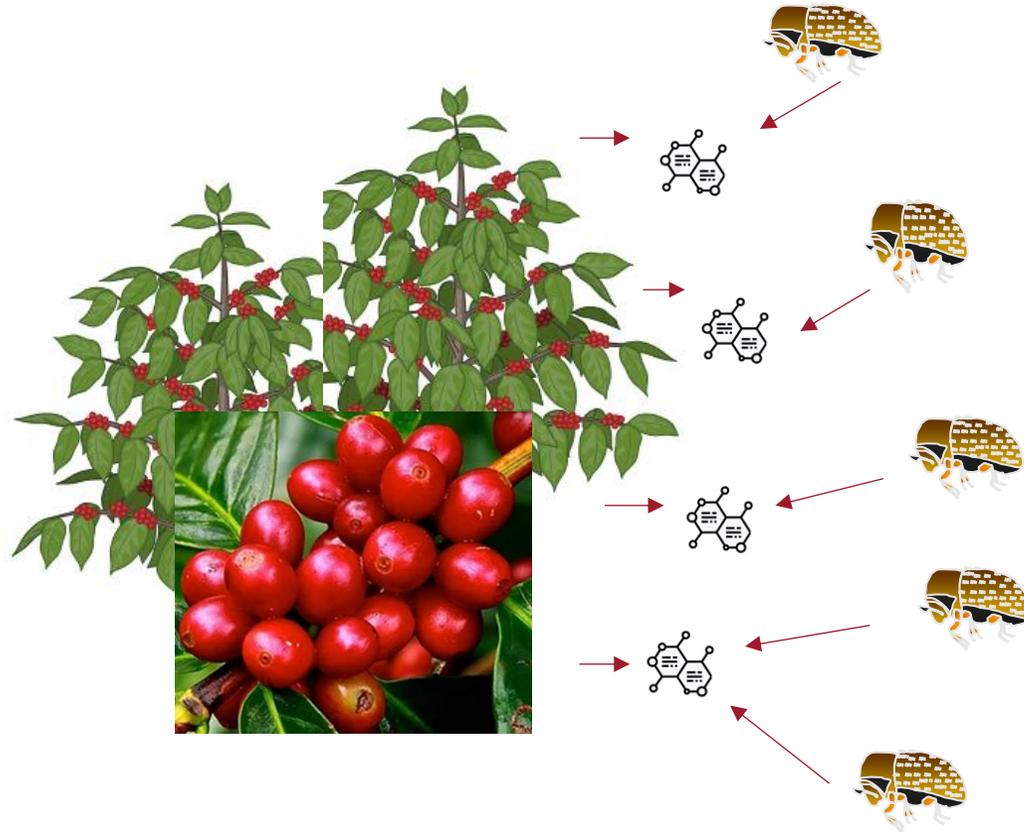
Silenciamiento de Genes en café-

Objetivo: entender la comunicación café-broca y conferir resistencia al insecto



Frutos producen volátiles que atraen la plaga.
Ninguna estrategia de control evita que el insecto llegue y ataque el fruto

Estudios comunicación café-broca



- Idárraga, S. M., Castro, A. M., Macea, E. P., Gaitán, A. L., Rivera, L. F., **Cristancho**, M. A., & **Góngora**, C. E. (2012). Sequences and transcriptional analysis of *Coffea arabica* var. Caturra and *Coffea liberica* plant responses to coffee berry borer *Hypothenemus hampei* (Coleoptera: Curculionidae: Scolytinae) attack. *Journal of Plant Interactions*, 7(1), 56-70.

Crispr Cas/9: Tecnología de silenciamiento

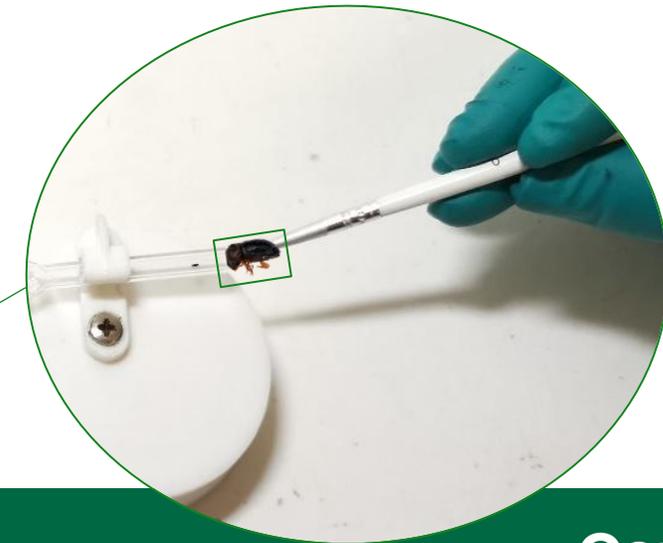
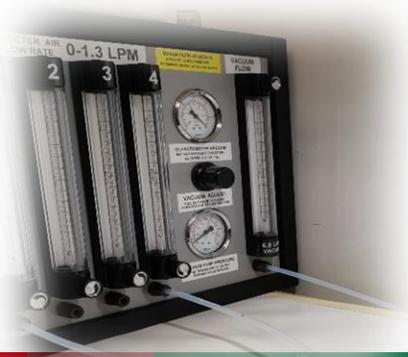
Plantas seleccionadas

Evaluación de los frutos frente a la broca – olfatometría

Disminución en la emisión de volátiles

Disminuye la atracción de la broca (50%- 75%)- Menor infestación

Prueba de concepto de silenciamiento en café



¡Muchas Gracias!

Para superar los desafíos del cultivo del café—clima, plagas y enfermedades—necesitamos de la biología molecular.

El futuro del café se escribe a nivel molecular!

