University Gent „Cocoa Workshop“

Physicochemical networks during cocoa fermentation

Daniel Kadow

Gent

September 2nd 2013

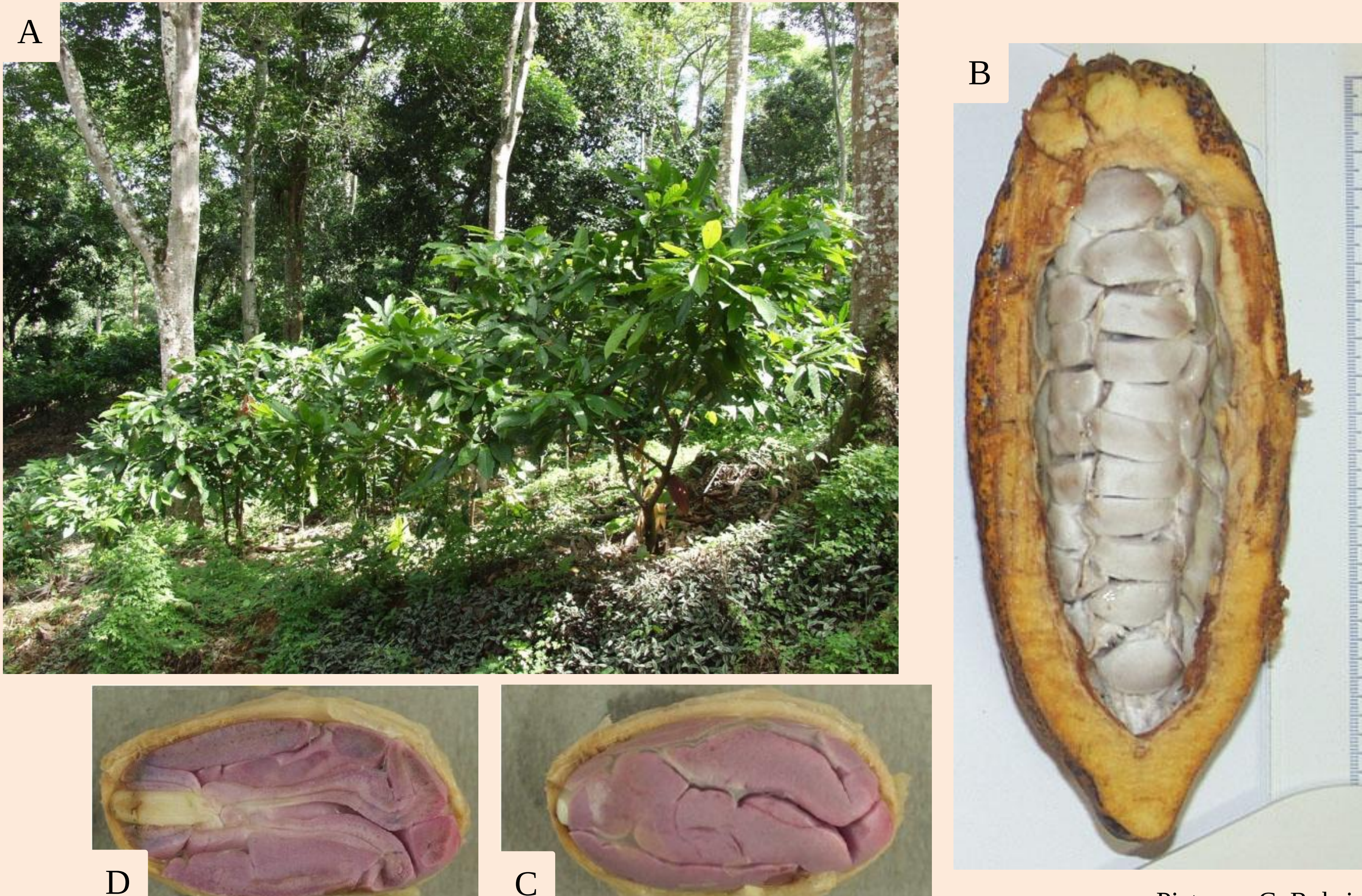
The Cocoa Tree **Chocolate Manufacturing**

A

B

C

Pictures: C. Rohsius and D. Kadow



Pictures: C. Rohsius

The Cocoa Tree — Distribution

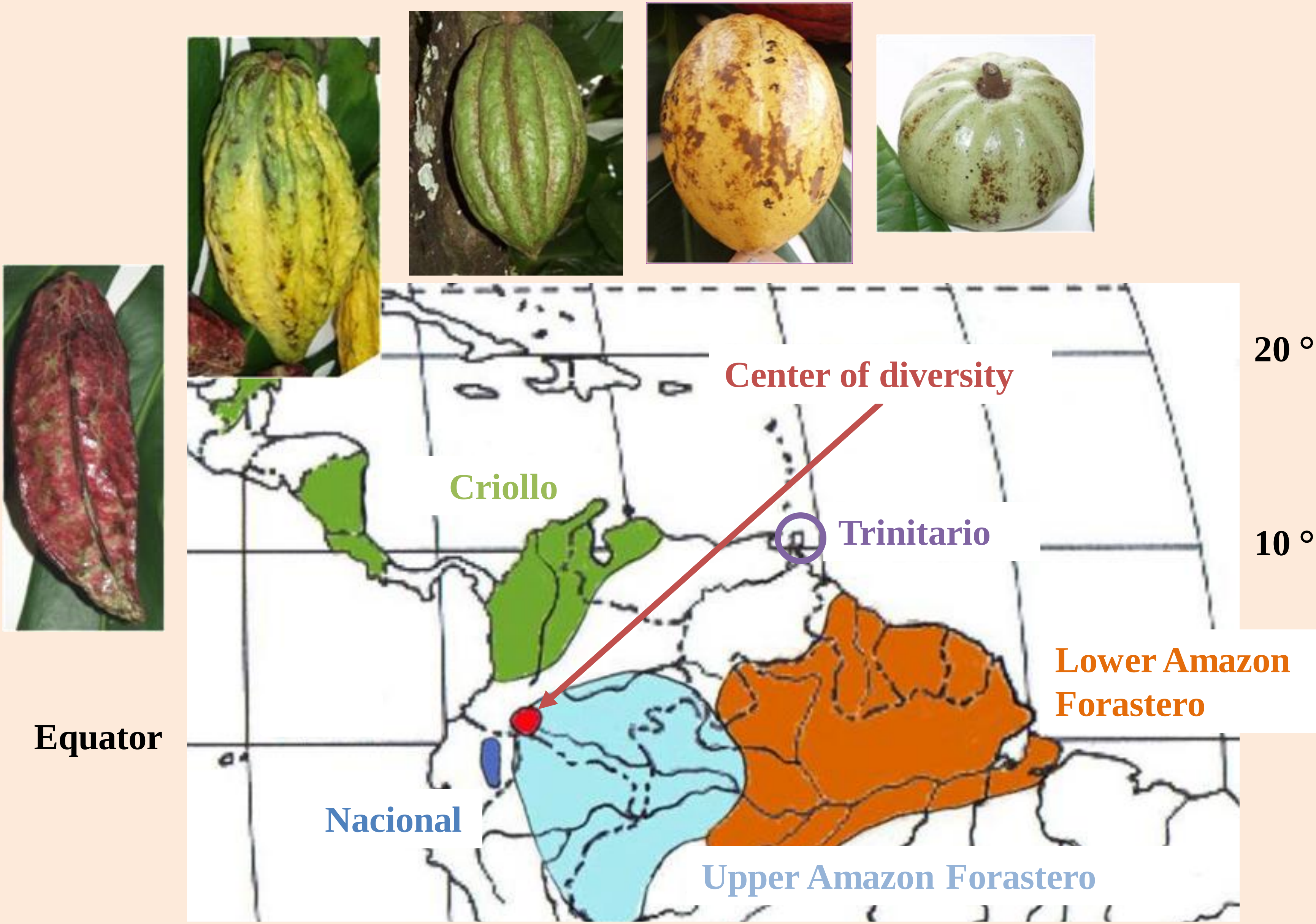


Figure: according to Parra 2000, Bartley 2005 and Rohsius 2007; pictures C. Rohsius

Figure: according to Parra 2000, Bartley 2005 and Rohsius 2007; pictures S. Elwers

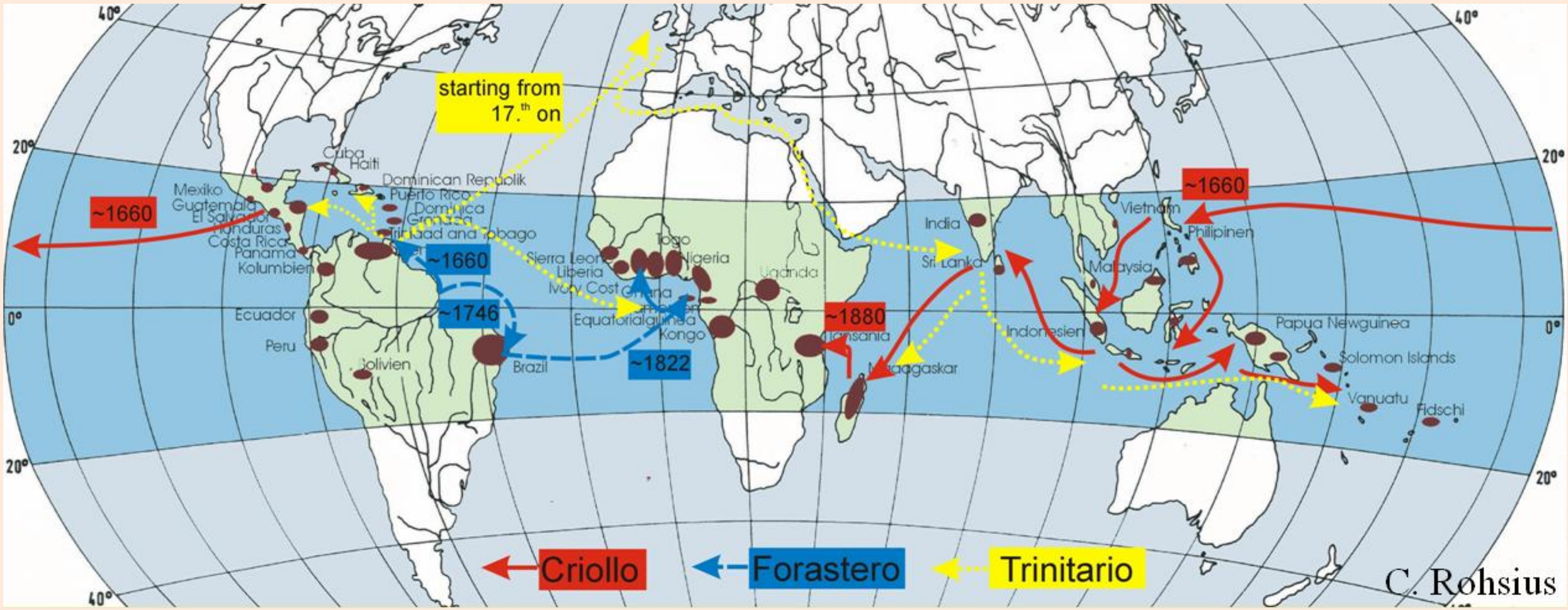
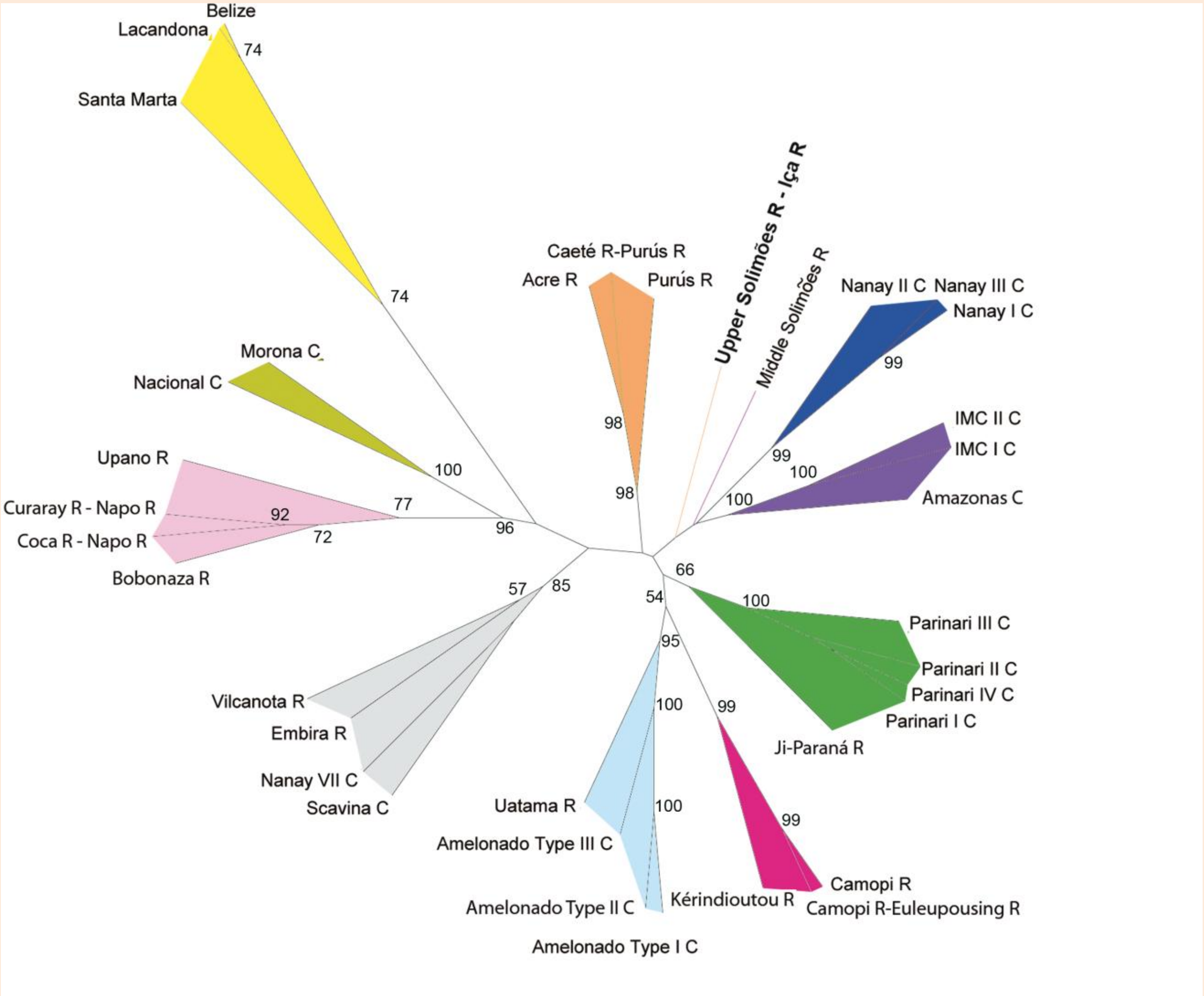


Fig. C. Rohsius



Colors indicate the inferred genetic cluster to which the subcluster belongs: Marañon (●), Guiana (●), Contanama (●), Curaray (●), Nanay (●), Iquitos (●), Nacional (●), Purús (●), Criollo (●), and Amelonado (●),(C=Clones; R=River).

Fig. Motamayor et al., 2008

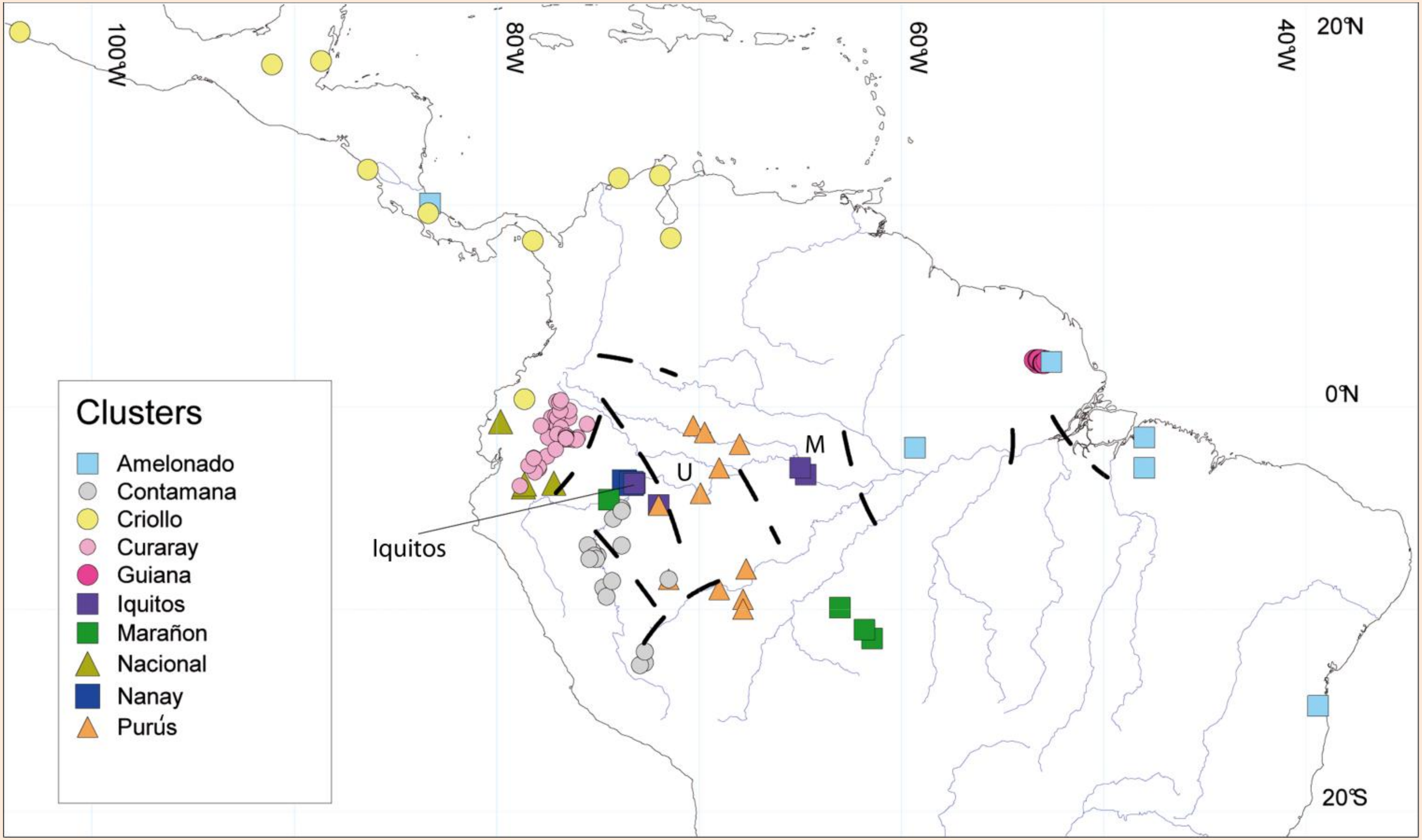
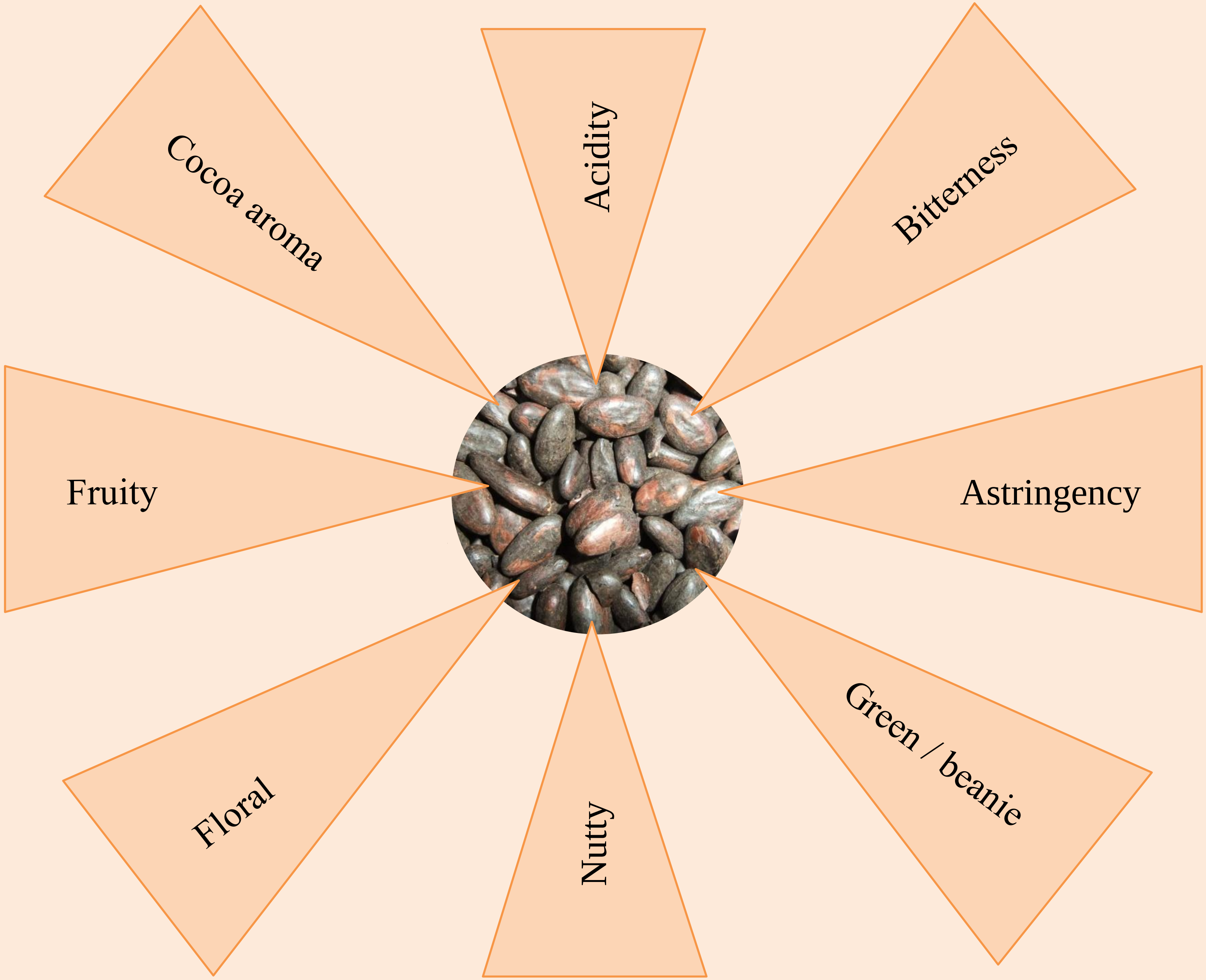


Fig. Motamayor et al., 2008

Aroma / Taste Attributes



Sensory characteristics of fresh cocoa seeds

A) No cocoa aroma

B) Astringent and bitter taste

C) No storage stability

Picture: http://www.infozentrum-schoko.de/auf-der-kakaoplantage.html

Cocoa — Post Harvest Treatment

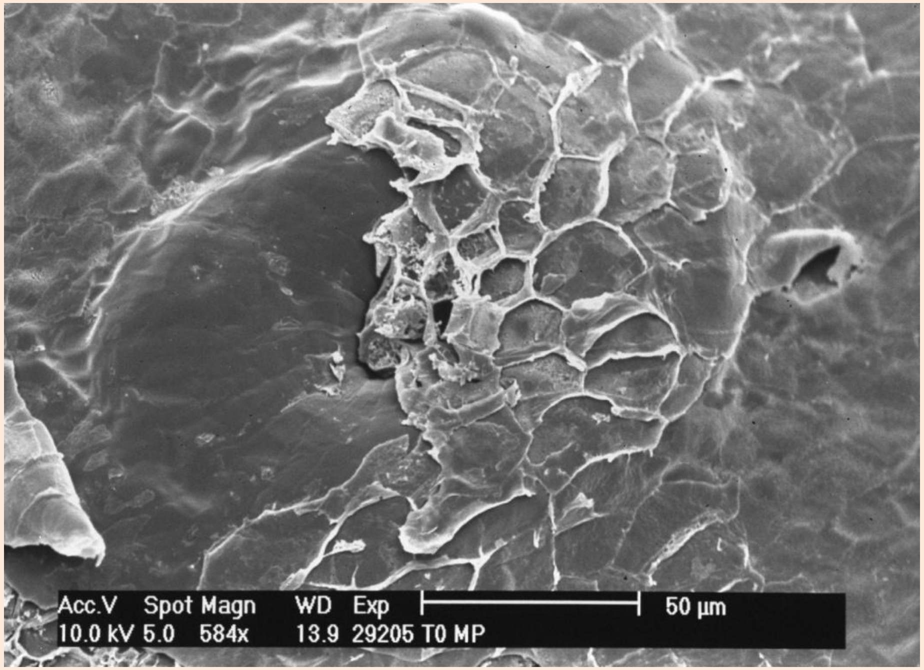
Pictures: http://www.infozentrum-schoko.de/auf-der-kakaoplantage.html; C. Rohsius and D. Kadow

30 - 45°C | **50 °C**

1.1. Anaerobic Phase	**1.2. Microaerobic Phase**	**2. Aerobic Phase**
Yeast dominated	Lactic acid bacteria dominated	Acetic acid bacteria dominated
Ethanol and Pectinase	Lactic acid	Acetic acid

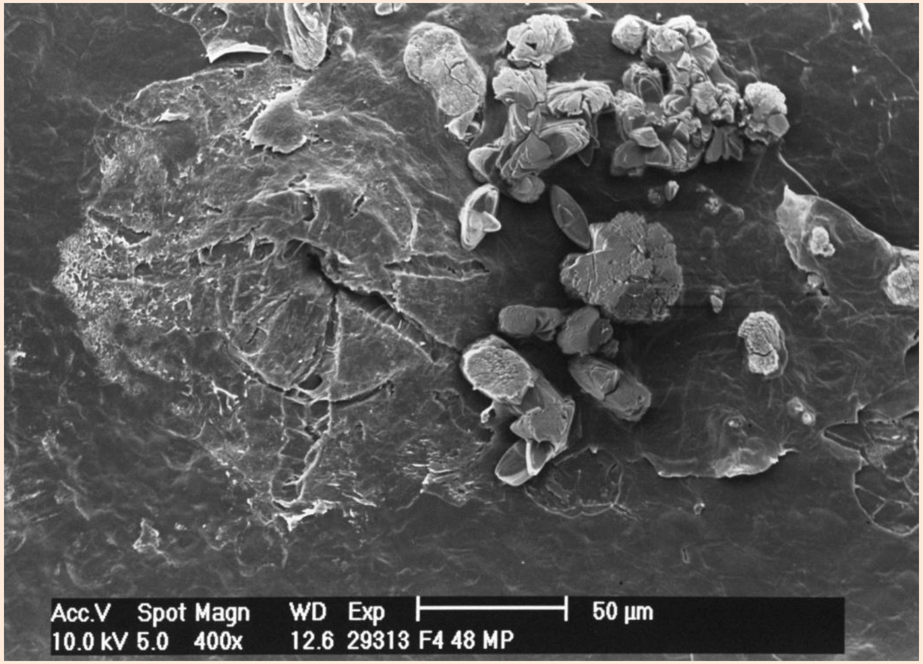
Cocoa Aroma Precursor formation

Figure: D. Kadow

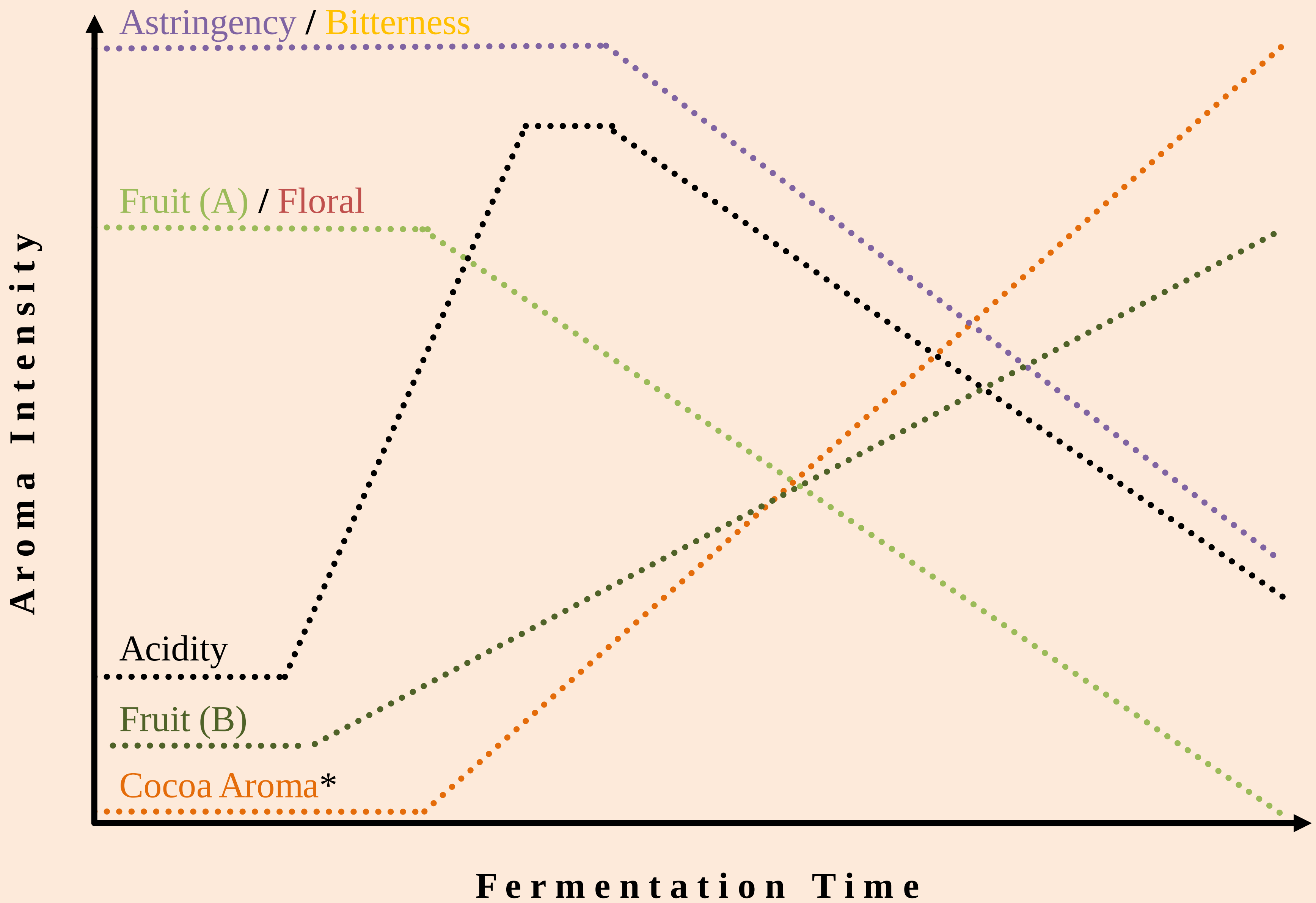


Micropyle of a fresh seed

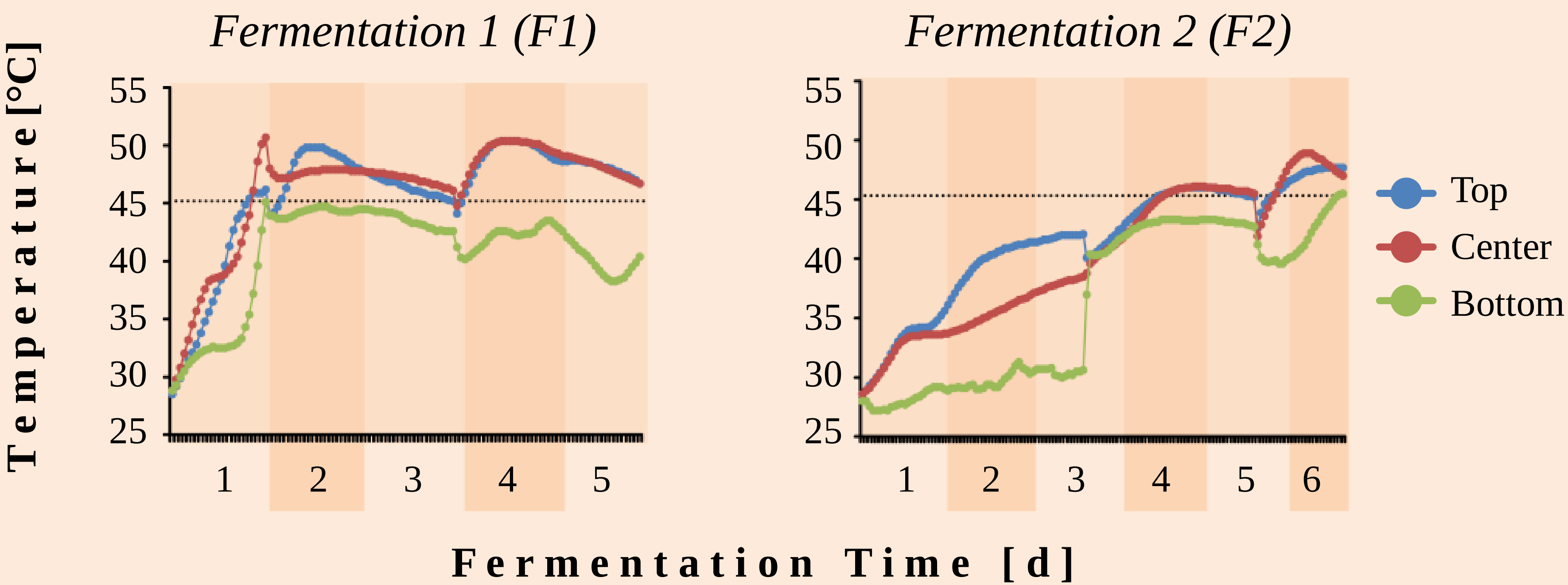
Micropyle of a fermenting seed



Pictures C. Bahmann

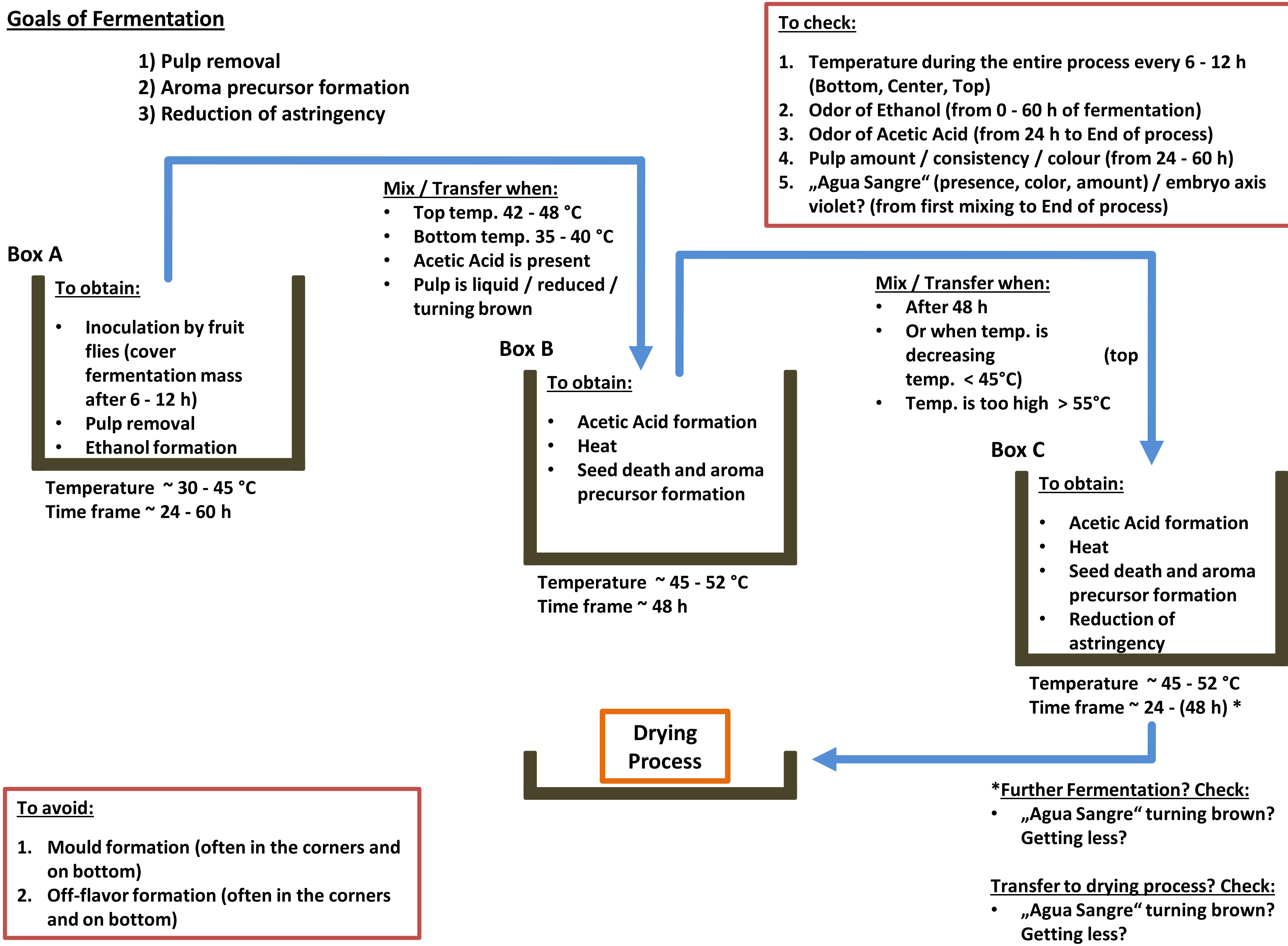


*After drying and roasting

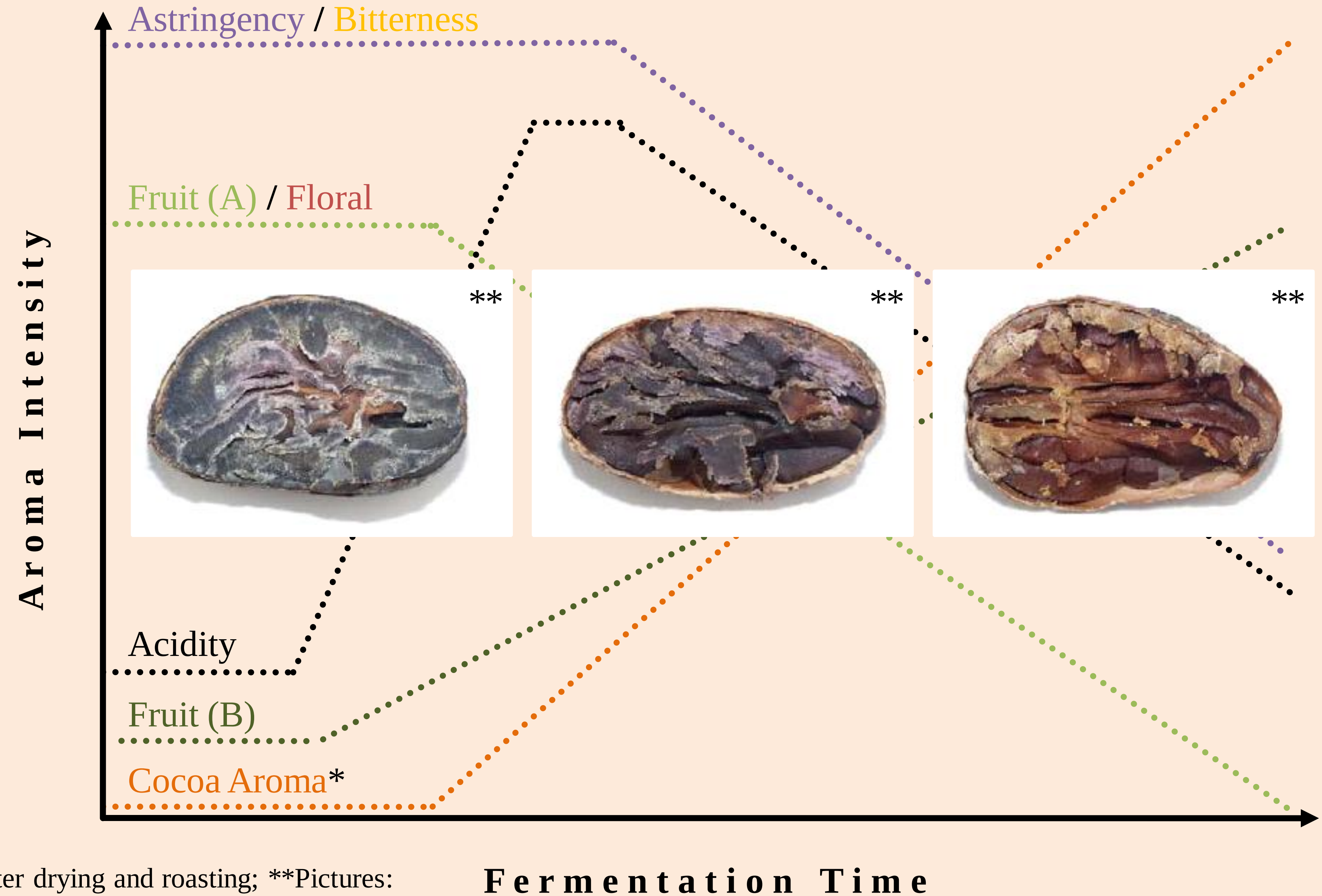


	F1	F2
Fermented	36%	14%
Partially fermented	38%	36%
Under-fermented	26%	49%

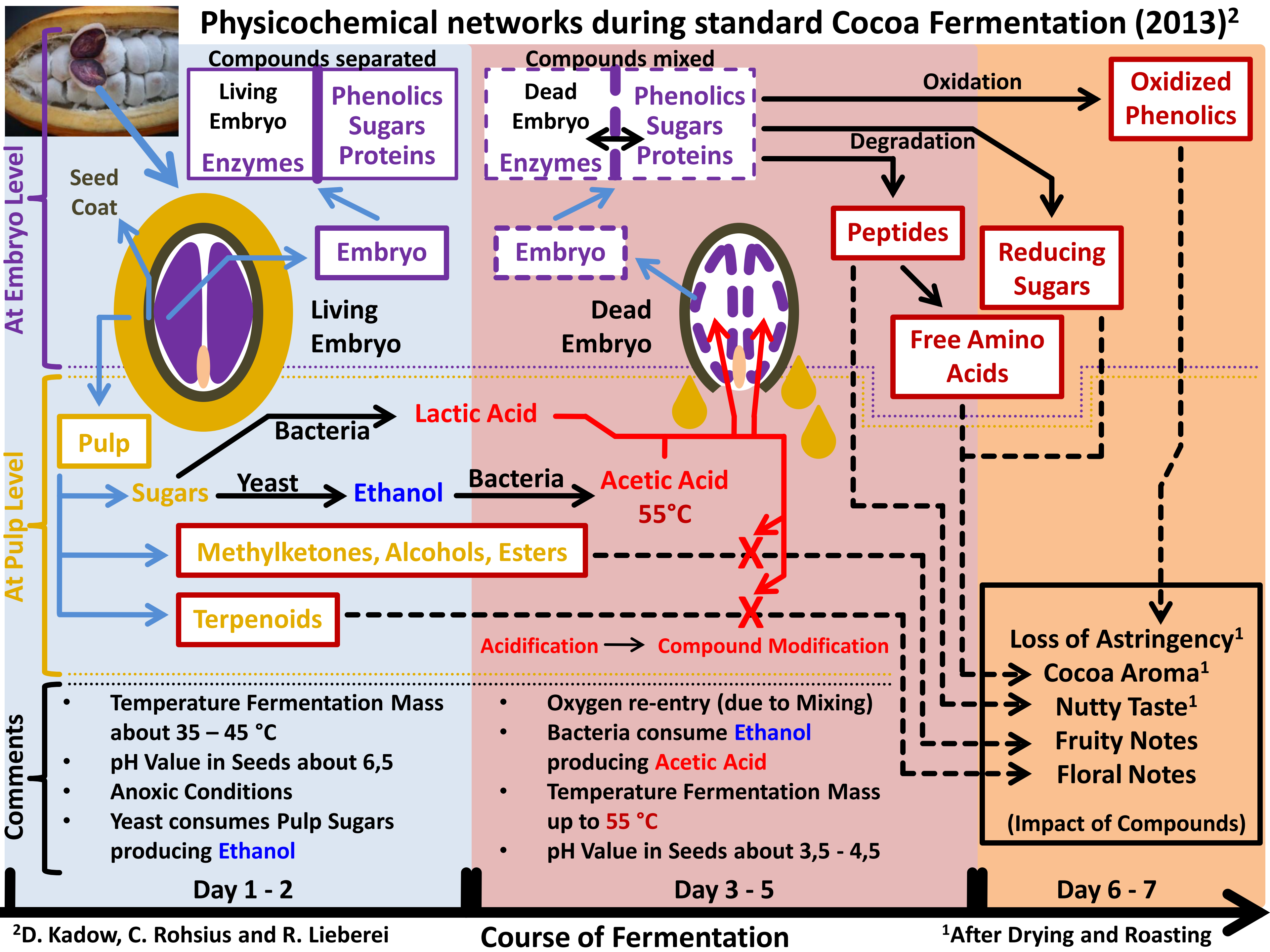

Pulp amount
Fermentation box size
Fermentation box material
Fermentation box insulation
Climate
Box enclosure
Time of transportation
?
Transport containers
Pathogenic fungi
Inoculation
Yeast inhibitors
Seed-own Pectinase
Genotype
Pulp pectine content
Pulp sugar content
Acidity of the Pulp
Pulp pH



Fermentation Management Scheme Ecuador, University of Hamburg, Version 2013, D. Kadow



*After drying and roasting; **Pictures: „Cocoa Cut Test Chart“ (UHH and UWI)

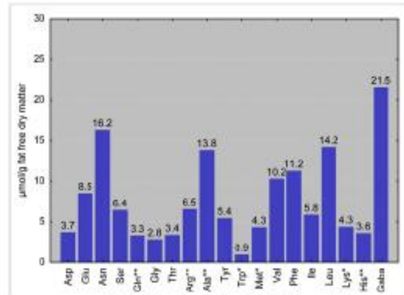

Physicochemical networks during standard Cocoa Fermentation (2013)²
At Embryo Level
At Pulp Level
Comments
Compounds separated
Living Embryo
Enzymes
Phenolics
Sugars
Proteins
Seed Coat
Embryo
Living Embryo
Compounds mixed
Dead Embryo
Enzymes
Phenolics
Sugars
Proteins
Embryo
Dead Embryo
Oxidation
Oxidized Phenolics
Degradation
Peptides
Reducing Sugars
Free Amino Acids
Pulp
Bacteria
Lactic Acid
Sugars
Yeast
Ethanol
Bacteria
Acetic Acid 55°C
Methylketones, Alcohols, Esters
Terpenoids
Acidification
Compound Modification
Loss of Astringency¹
Cocoa Aroma¹
Nutty Taste¹
Fruity Notes
Floral Notes
(Impact of Compounds)
• Temperature Fermentation Mass about 35 – 45 °C
• pH Value in Seeds about 6,5
• Anoxic Conditions
• Yeast consumes Pulp Sugars producing Ethanol
• Oxygen re-entry (due to Mixing)
• Bacteria consume Ethanol producing Acetic Acid
• Temperature Fermentation Mass up to 55 °C
• pH Value in Seeds about 3,5 - 4,5
Day 1 - 2
Day 3 - 5
Day 6 - 7
²D. Kadow, C. Rohsius and R. Lieberei
Course of Fermentation
¹After Drying and Roasting

Menu — Comparison of Samples

1 | Trinidad | Sample_5 | Picture_2

2 | Trinidad | Sample_5 | Free Amino Acid

Amino Acids



Contents of amino acids [µmol/g fat free dry matter]
*Trp, *Met, *Lys: detected in selected samples only,
**Gln, **His and **Arg, **Ala given as sum where separate detection failed

	fat free dry matter[1)]
nitrogen	4.06 %
raw protein[2)]	4.46 %

[1)] w/w in fat free dry matter = dry, defatted, deshelled fermented nibs (cotyledons only)
[2)] raw protein is calculated as: (nitrogen content - (caffeine nitrogen + theobromine nitrogen) x 6.25

Sample analysed in 2010

3 | Trinidad | Sample_5 | Further Compounds

Further Compounds

	compound	fat free dry matter[1)]
acids	acetic acid lactic acid	0.2 % 1.2 %
methylxanthines	theobromine caffeine	2.12 % 0.61 %
phenolic substances	total phenolics (Folin) epicatechin catechin cyanidin-3-arabinoside cyanidin-3-galactoside	79.5 ‰ 11.01 ‰ 0.12 ‰ 0.48 ‰ 0.16 ‰
fermentation index		0.68
reducing sugars	sucrose fructose glucose	0.60 % 0.23 % < 0.1 %

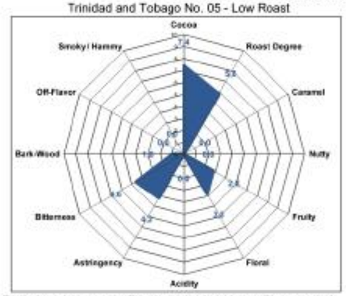
nc not calculated; na not analysed
[1)] w/w (or as stated) in fat free dry matter = dry, defatted, deshelled fermented nibs (cotyledons only)

Sample analysed in 2010

4 | Trinidad | Sample_5 | Aroma Description

Aroma Description

Trinidad and Tobago No. 05 - Low Roast



Rich chocolate aroma. First taste is chocolate with some dark fruit notes--raisin and some prune. There is also just an edge of a slightly under ripe banana. Good dark wood presence with rich browned notes. Very clean and clear oveall flavor. (Low Roast: 121°C, 25 min*

*We thank Edward Seguine (Mars Inc.) for his skilled contribution with respect to this aroma description

From: Cocoa Atlas 2010 Edition

Bulk cocoa

95 % of world wide production

Strong chocolate aroma

No special aroma notes

Endogenous formation by storage compound degradation
(e.g. proteins and carbohydrates)

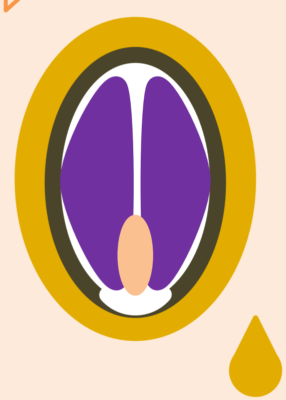
Fine cocoa

5 % of world wide production

Weak chocolate aroma

Special aroma notes (fruity and floral)

Migration from pulp to cotyledon tissue



Bulk cocoa

CCN 51

Raw cocoa

No special aroma notes

Pulp

Astringent, acid, no special aroma notes

Fine or flavor cocoa

EET 62

Raw cocoa

Fruity and floral aroma notes

Pulp

Fruity and floral aroma notes

SCA 6

Raw cocoa

Floral aroma notes

Pulp

Sweet, floral, fruity aroma notes

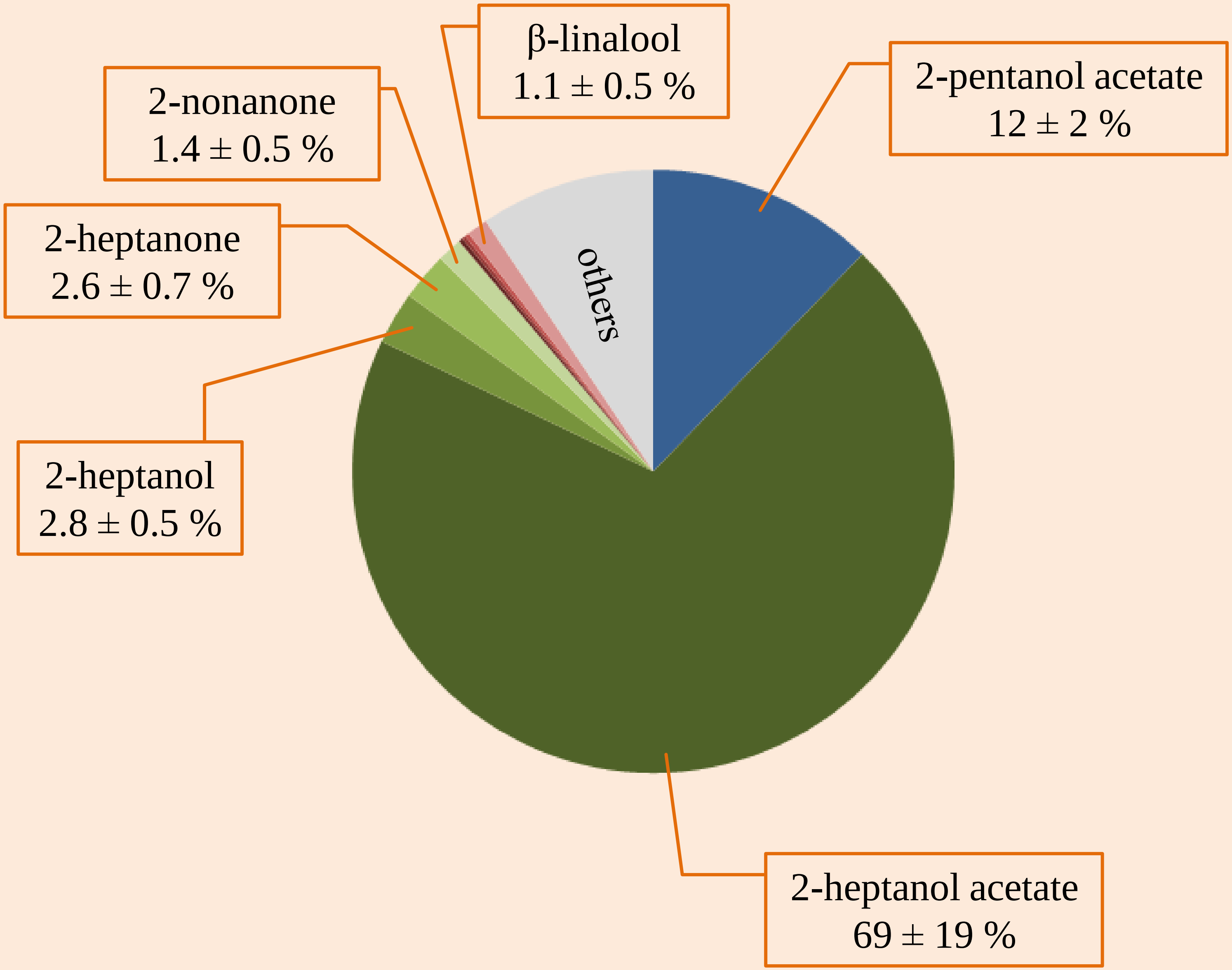
Pictures D. Kadow

Questions

1. Do CCN 51, EET 62 and SCA 6 differ regarding the pulp volatile components?

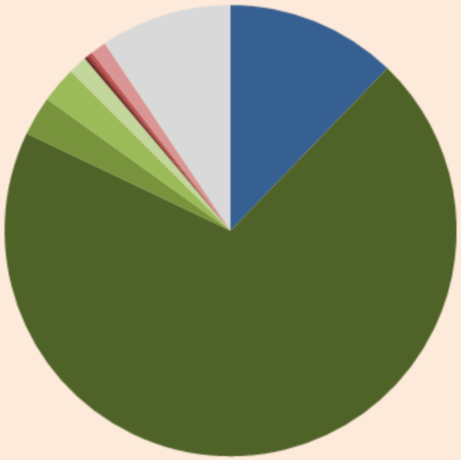
2. Do potential differences match the organoleptic descriptions given by Eskes *et al.*?

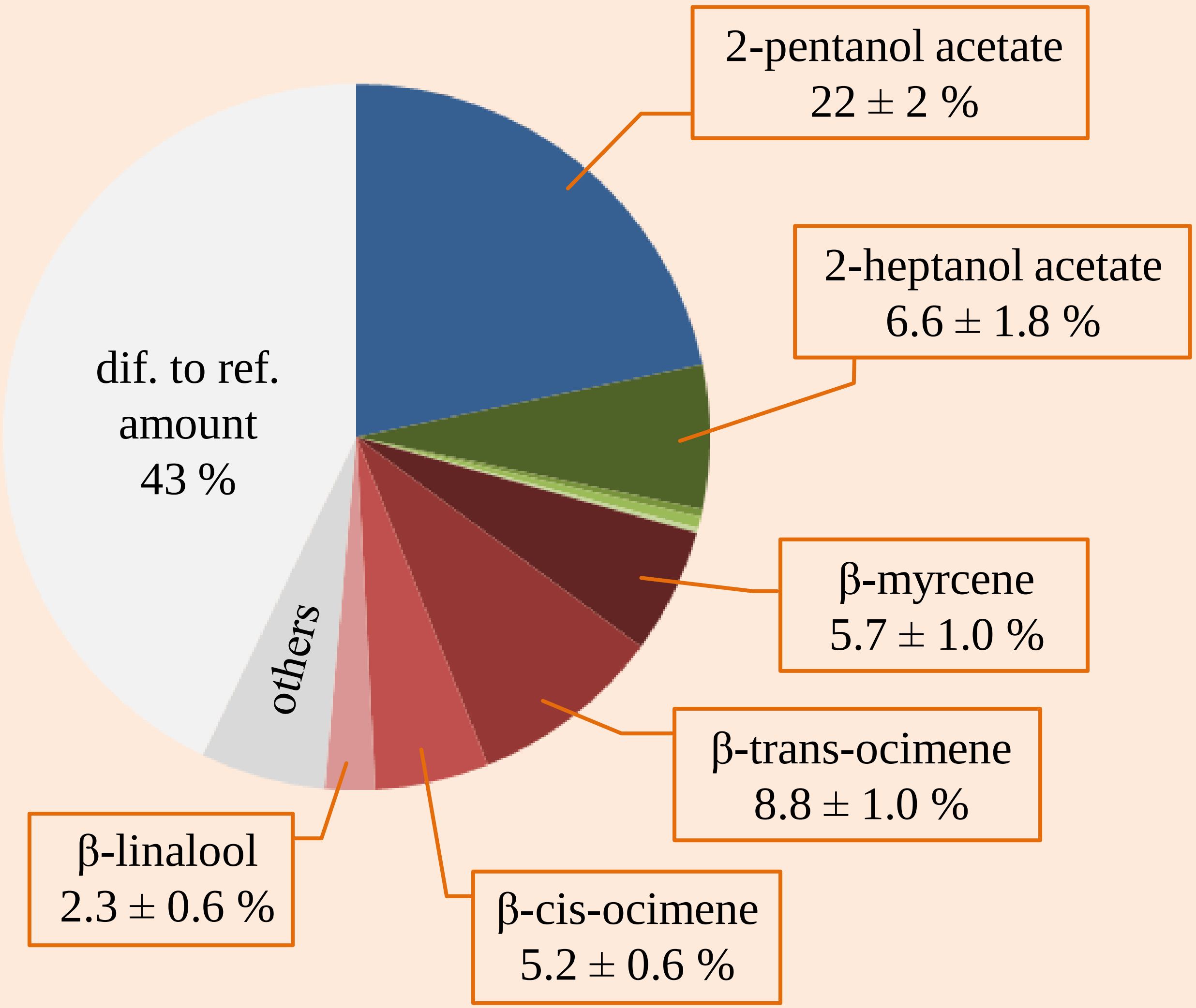
EET 62 pulp volatile composition



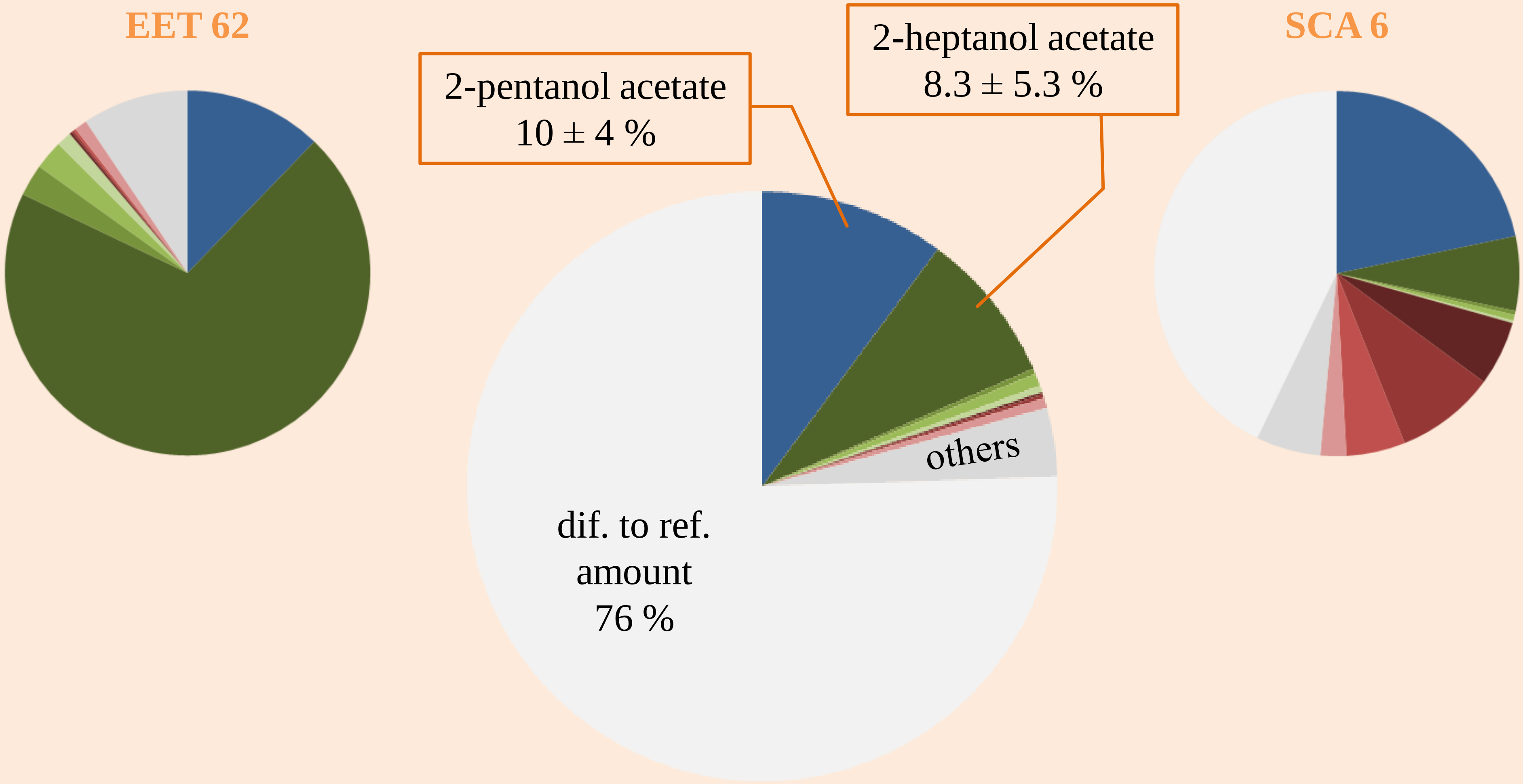
Kadow *et al.*, 2013

EET 62

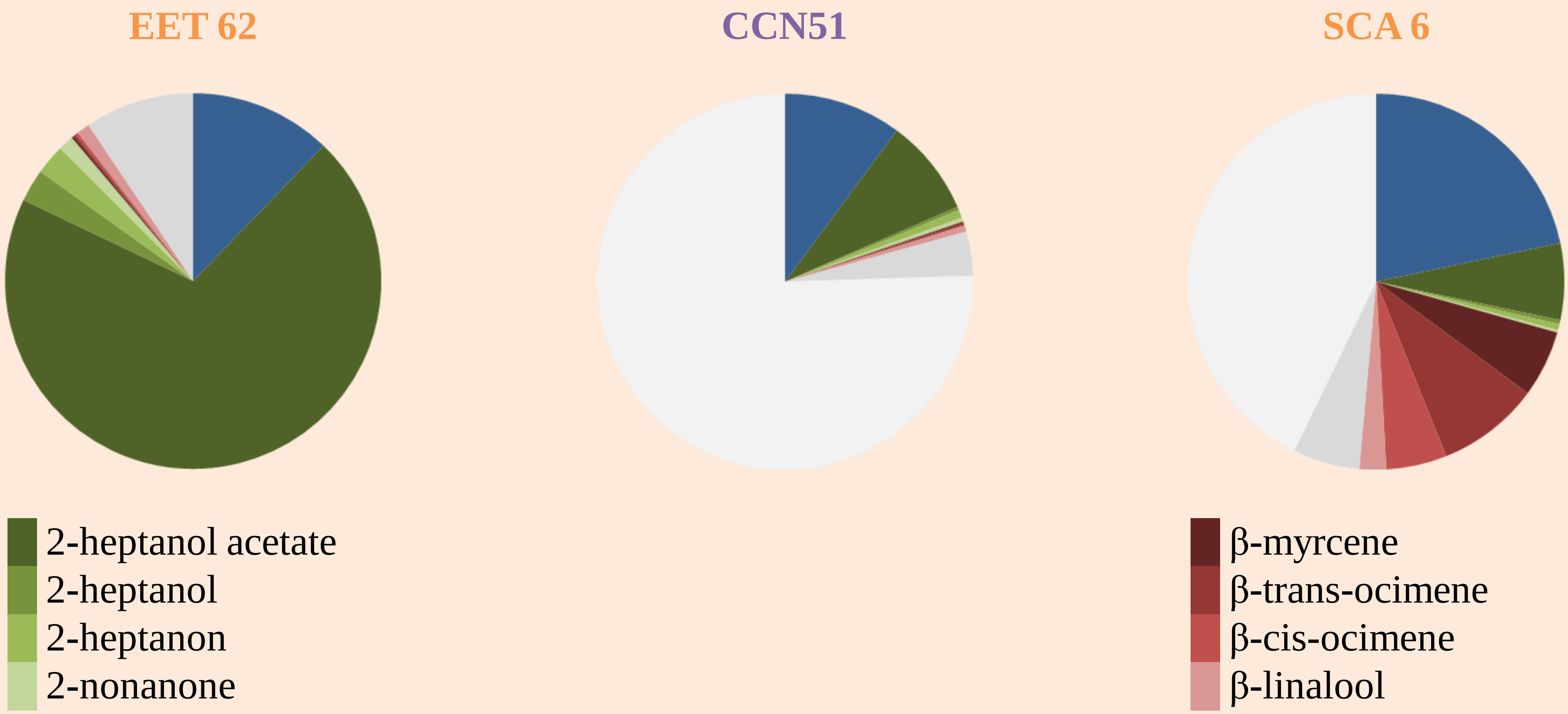




Kadow *et al.*, 2013



Kadow *et al.*, 2013

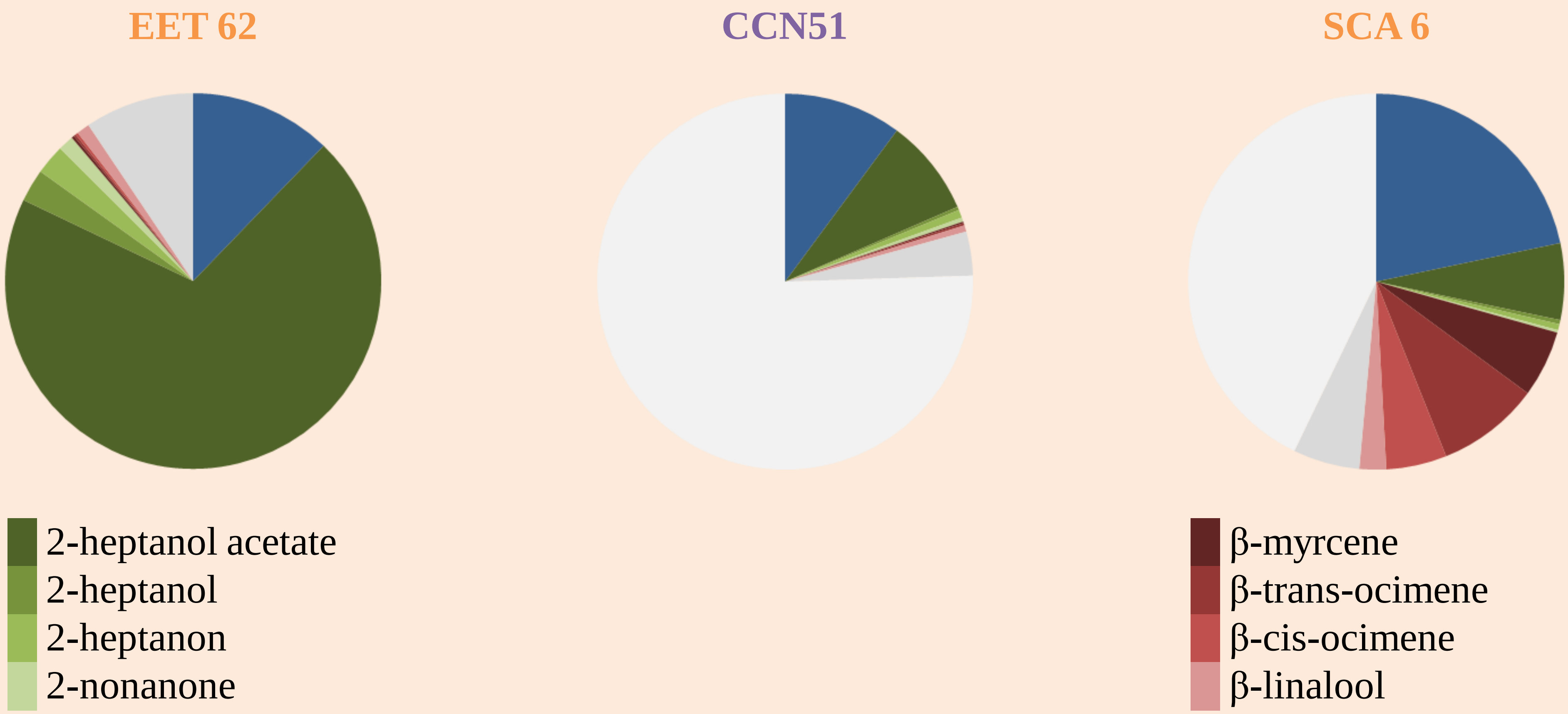


1. Do CCN 51, EET 62 and SCA 6 differ regarding the pulp volatile components?

Kadow *et al.*, 2013

Results

Fine or flavor **components**



2. Do potential differences match the organoleptic descriptions given by Eskes *et al*.?

Kadow *et al*., 2013

EET 62	odor type[1]	odor description[1]
2-heptanol acetate	brown	fruity
2-heptanol	citrus	lemon grass, floral
2-heptanon	cheesy	fruity, coconut
2-nonanone	fruity	fresh, sweet

SCA 6	odor type[1]	odor description[1]
β-myrcene	spicy	balsamic, citrus
β-trans-ocimene	herbal	licorice, sweet
β-cis-ocimene	floral	flower, sweet
β-linalool	floral	citrus, sweet

Kadow *et al.*, 2013; [1]according to Luebke, 2011

Summary and discussion

1. CCN 51, EET 62 and SCA 6 differ regarding the pulp volatile components. β-myrcene, β-trans-ocimene, β-cis-ocimene and β-linalool are characteristic for SCA 6. Regarding EET 62 2-heptanol, 2-heptanol acetate, 2-heptanone and 2-nonanone are typic.

2. The organoleptic properties of the individual substances match the descriptions given for the pulp by Eskes *et al*., 2007.

We conclude that the above mentioned molecules are the main components of SCA 6 and EET 62 fine aroma.

Accordingly, fine aroma components apparently derive from different metabolic pathways depending on the genotype.

The Cocoa Tree
-
Prospects 2020

Daniel Kadow

Gent

September 2nd 2013

Quality

<u>Economical components</u>

- Stability of production
- Stability of food safety

<u>Ecological components</u>

- Species diversity
- Sustainability

<u>Social components</u>

- Farmer income
- Job safety

Witches´ broom disease

Decrease of raw cocoa production by 50-90%

1989

Raw cocoa prod. ~ 400 000 t

2000

~ 80 000 t

Map: Google Maps http://maps.google.de/maps?hl=de&tab=wl

Disease control

<u>Chemical control</u>

i.e. application of pesticides

<u>Phytosanitation</u>

i.e. removal of infested material

<u>Biological control</u>

i.e. application of antagonistic fungus

<u>Genetic resistance</u>

i.e. breeding of resistant clones

Cocoa genotype	Present rating
TSH 979	R
TSH 1104	MR
SCA 3	R
SCA 5	R
SCA 6	HR
SCA 7	MR
SCA 8	R
SCA 9	HR
SCA 10	HS
SCA 11	HR
SCA 12	HR

Present status of witches' broom disease of cocoa in Trinidad

(*Keywords: Crinipellis perniciosa*, witches' broom disease, cocoa, Trinidad)

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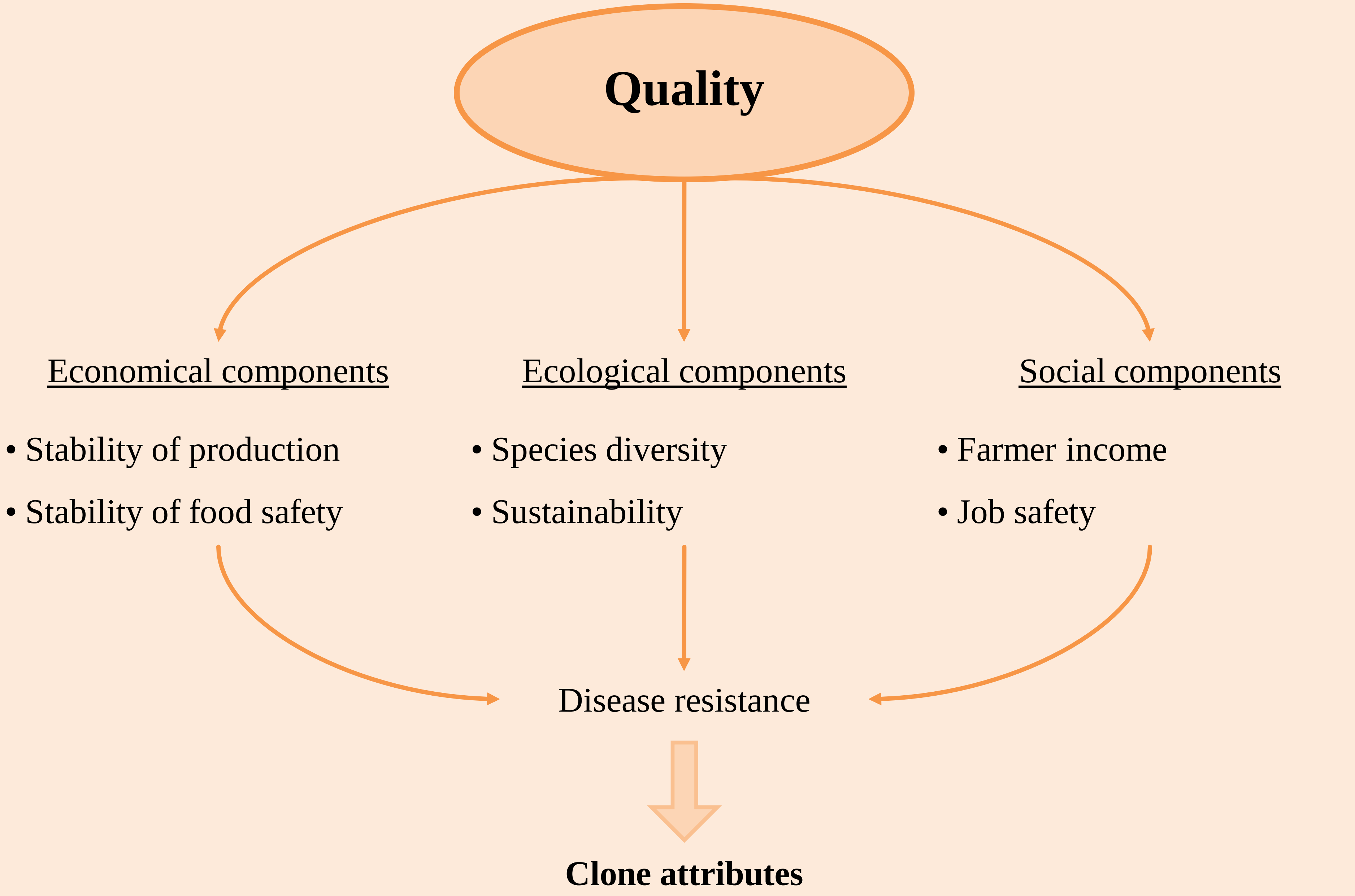

Quality
Economical components
• Stability of production
• Stability of food safety
Ecological components
• Species diversity
• Sustainability
Social components
• Farmer income
• Job safety
Disease resistance
Clone attributes

The Cocoa Tree

Clone Attributes

Disease resistance

Butter fat content

Yield

Fine aroma

Flower set

Drought tolerance

*Picture: Phillips and Wilkinson 2007. *Phytopathology*, 97 (12)
**Pictures: B. Rudolph, Universität Hamburg, Germany.

Disease resistance

Mapping QTLs for Resistance to Frosty Pod and Black Pod Diseases and Horticultural Traits in *Theobroma cacao* L.

J. Steven Brown,* Wilbert Phillips-Mora, Emilio J. Power, Cheryl Krol, Cuauhtemoc Cervantes-Martinez, Juan Carlos Motamayor, and Raymond J. Schnell

Mapping QTLs for Witches' Broom (*Crinipellis perniciosa*) Resistance in cacao (*Theobroma cacao* L.)

Fábio Gelape Faleiro[1,2], Vagner Tibaldi Queiroz[3], Uilson Vanderlei Lopes[1,*], Cláudia Teixeira Guimarães[3], José Luis Pires[1], Milton Macoto Yamada[1], Ioná Santos Araújo[4], Messias Gonzaga Pereira[4], Raymond Schnell[5], Gonçalo Apolinário de Souza Filho[4], Cláudia Fortes Ferreira[1], Everaldo Gonçalves Barros[3] & Maurílio Alves Moreira[3]

Mapping of Quantitative Trait Loci for Butter Content and Hardness in Cocoa Beans (*Theobroma cacao* L.)

Ioná S. Araújo · Gonçalo A. de Souza Filho · Messias G. Pereira · Fábio G. Faleiro · Vagner T. de Queiroz · Cláudia T. Guimarães · Maurílio A. Moreira · Everaldo G. de Barros · Regina C. R. Machado · José L. Pires · Raymond Schenell · Uilson V. Lopes

A genomewide admixture mapping study for yield factors and morphological traits in a cultivated cocoa (*Theobroma cacao* L.) population

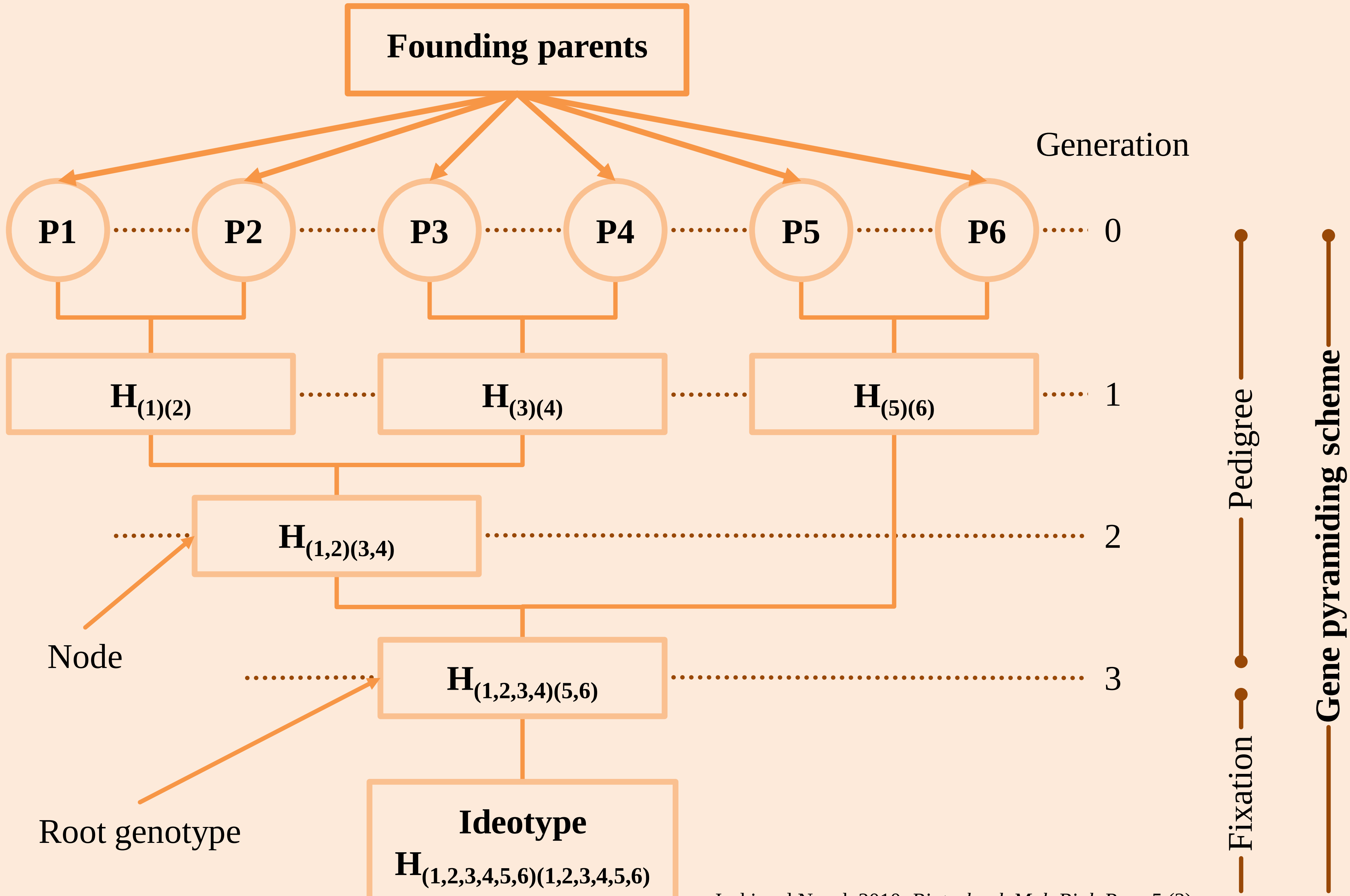
Maria Marcano · Sonia Morales · Maria Theresa Hoyer · Brigitte Courtois · Ange Marie Risterucci · Olivier Fouet · Tatiana Pugh · Emile Cros · Ventura Gonzalez · Manuel Dagert · Claire Lanaud

Yield

Fine aroma

Flower set

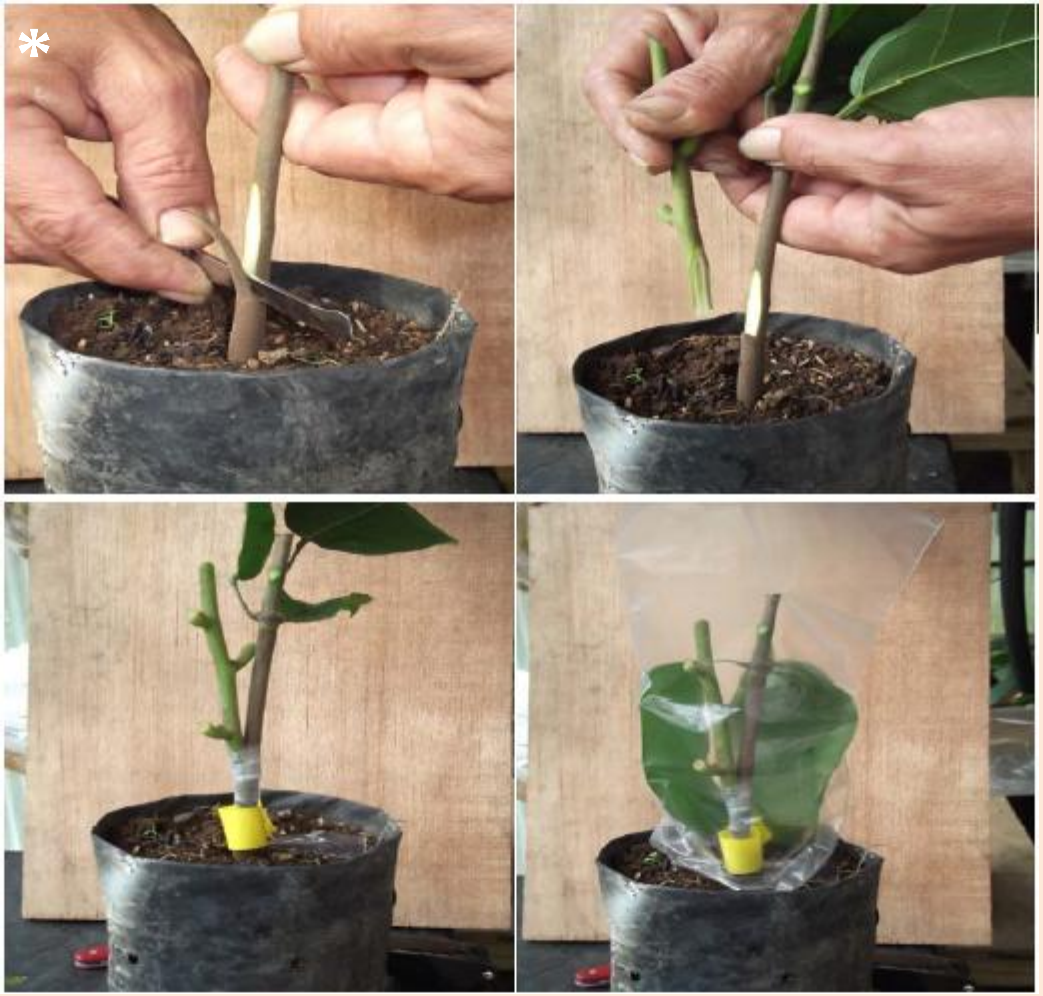
*Picture: Phillips and Wilkinson 20
**Pictures: B. Rudolph, Universität



Joshi and Nayak 2010. *Biotechnol. Mol. Biol. Rev.*, 5 (3)

Classical approaches:

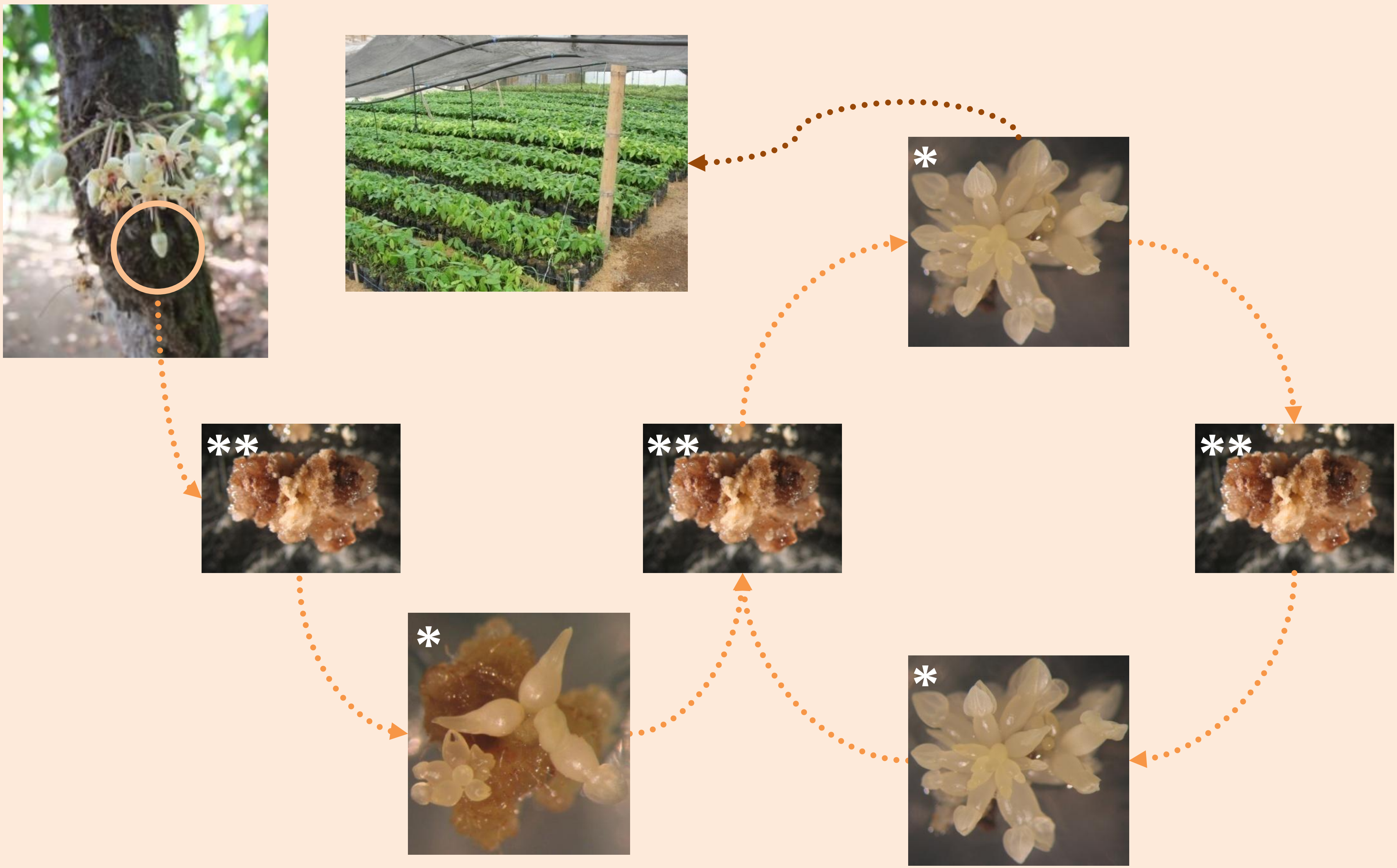
- Graftings
- Buddings
- Rooted cuttings



*Pictures: A. Sánchez, 2012. CATIE, Turrialba, Costa Rica.

Multiplication of elite clones

Multiplication of elite clones



*Pictures: F. Wuelfing, University of Hamburg, Germany. **Picture: T. Mueller, University of Hamburg, Germany.

<u>elite clones</u>

obtained through molecular breeding approaches

<u>clone propagation</u>

by classical and biotechnological methods

<u>rootstocks</u>

development and use of defined rootstocks

<u>phytosanitation</u>

removal of infested material

<u>agroforestry</u>

establishment of buffer areas and corredors

<u>regionality</u>

adaptation to local conditions

<u>biocontrol</u>

use of endophytic bacteria and fungi

elite clones

obtained through molecular breeding approaches

clone propagation

by classical and biotechnological methods

fermentation

optimized fermentation protocols and techniques

rootstocks

development and use of defined rootstocks

phytosanitation

removal of infested material

agroforestry

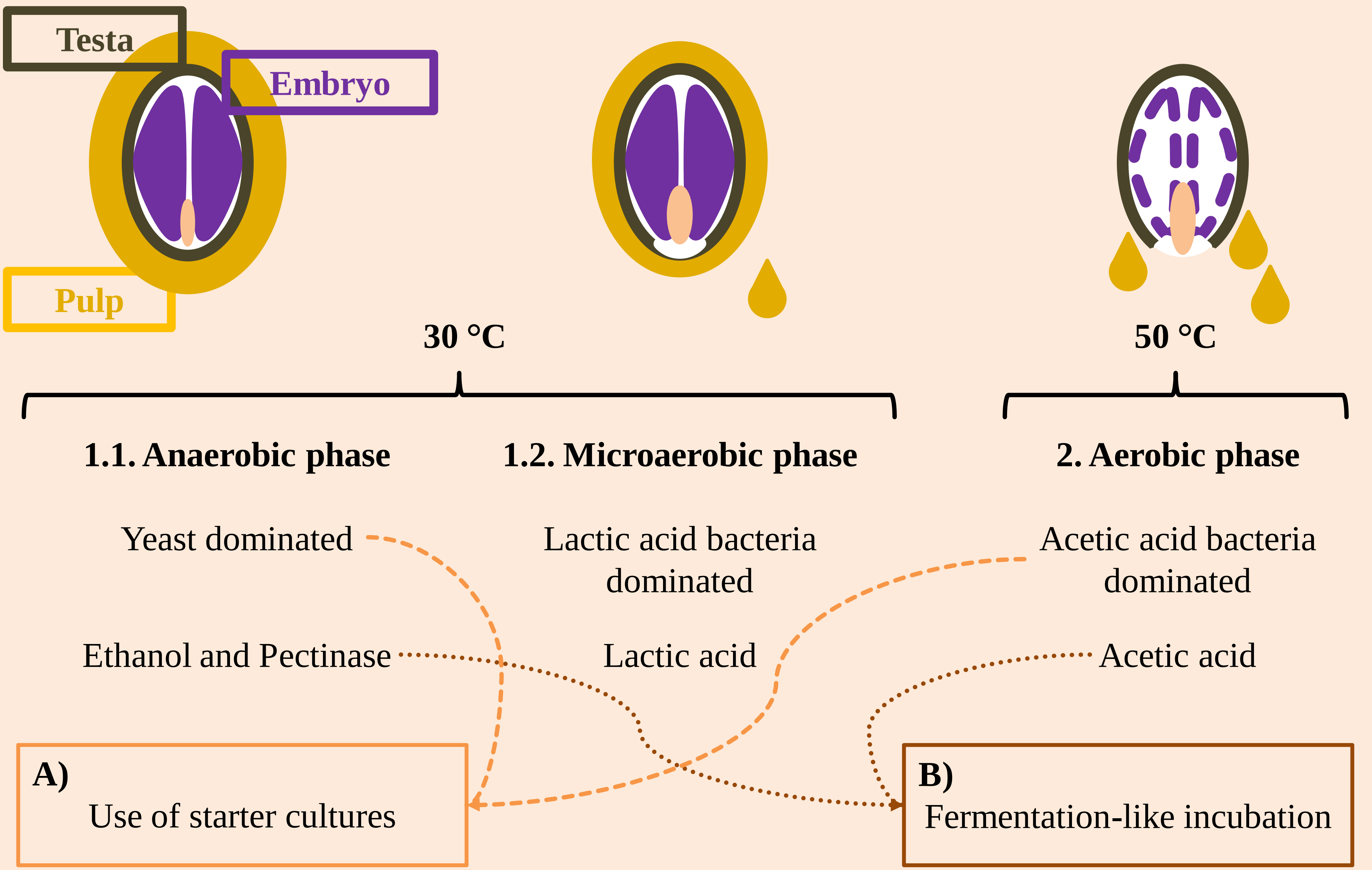
establishment of buffer areas and corredors

regionality

adaptation to local conditions

biocontrol

use of endophytic bacteria and fungi


Testa
Embryo
Pulp
30 °C
50 °C
1.1. Anaerobic phase
1.2. Microaerobic phase
2. Aerobic phase
Yeast dominated
Lactic acid bacteria dominated
Acetic acid bacteria dominated
Ethanol and Pectinase
Lactic acid
Acetic acid
A) Use of starter cultures
B) Fermentation-like incubation

A) Molecular breeding approaches facilitate the combination of multiple quality-related attributes in elite clones (e.g. disease resistance, yield, fine aroma).

B) *In vitro* mass propagation of elite clones has the potential to ensure the supply of sufficient planting material.

C) Integrated approaches may unite different techniques (e.g. molecular breeding, *in vitro* mass propagation, biological disease control, fermenter technology).

→

1) Enhanced raw cocoa **quality**

2) Enhanced raw cocoa **diversity**

Thank you for your attention!