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Biochemical Ecology

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Imagining the squadrons of bacteria, moulds, insects and grazing mammals (not to speak of human beings and their activities), which attack, feed on and destroy plants, we must wonder why the landscapes are not bare but still covered with a lush vegetation. Biochemical ecology (or chemical ecology) emerged from the discovery of the chemicals involved in the above-mentioned interactions between plants, animals and microorganisms.

Since Fraenkel's remarkable article (1959) regarding the raison d'être of the so-called secondary plant substances (Hartmann, 1996), scientists are aware that plants (and to a smaller extent also animals and bacteria) produce a vast array of substances not only to defend themselves against pathogens and predators but also to attract organisms for their own benefit. Today, biochemical ecology is an established field of natural sciences (for a comprehensive review see Harborne, 2001). It is conceivable that a better knowledge of such chemically mediated interactions will stimulate biocontrolled farming and assist also coffee growers in their fight against pests in the plantations, and thus – in the long term – will undoubtedly improve coffee quality, to which all, farmers, manufacturers and scientists are committed (Illy, 1997).

Before passing over specifically to biochemical ecology in coffee, two well-studied examples will serve to illustrate this rapidly emerging discipline of science. Many flowering species attract the pollinator, such as an insect (e.g. bee, butterfly), a bird (e.g. humming bird), or a mammal (e.g. bat), by odours and/or pigmentation. Additionally and very frequently, the morphology of the flower complies with the needs of a specific pollinator, for example by providing a

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landing platform for bees and bumblebees or a hanging thin throat for hummingbirds, etc.

Generally, the pollinator is rewarded by nectar and by a more or less welcome dust of pollen. However, about one-third of all orchids do not invest energy into nectar production but have evolved flowers simulating various kinds of rewards. For example, several species mimic by odours, colours, shape and texture the female of a distinct solitary bee. The delusion is so accurate that the male tries to copulate with the flower whereby the entire pollinia are positioned, for example, on his head to carry them to the place of a subsequent frustrating pseudocopulation, finally resulting in the pollination of that flower (Schiestl et al., 1999).

And moreover, after pollination the flower produces a volatile substance usually emitted by non-receptive female insects to inhibit copulation (Schiestl and Ayasse, 2001). This example nicely illustrates that biochemistry (odours and pigmentation) of biochemical ecology cannot be separated from physical factors including morphology, texture, mechanical strength, nutritional energy and, last but not least, appearance in space and time.

A second example demonstrates how the plant calls for help when attacked by a predator. The larvae of the beet army worm, Spodoptera exigua, are a pest of the maize plant. In a series of brilliantly designed experiments, it has been shown that the saliva of the larvae contains a signal compound (perhaps partly originating from the destroyed plant membranes) that, locally, and later also systemically, induces the formation of leaf volatiles specifically attracting parasitic wasps (Cotesia marginiventris) which in turn lay their eggs into the larvae, and thus eliminate the predating insect (Turlings et al., 1990; Alborn et al., 1997). Additionally, the volatiles may protect the plant from attack by other herbivores (Kessler and Baldwin, 2001). Today, various examples of such interactions within – at least – three levels (tritrophic interaction: plant/predator/parasite) are known and have been successfully studied by coupling highly sensitive electrophysiological techniques with GC-MS (for reviews see: Dicke and Van Loon, 2000; Pichersky and Gershenzon, 2002).

Yet, secondary compounds govern, besides the sophisticated interactions described above, another key process of plant survival, which is named chemical defence (reviewed in Edwards, 1992; Harborne, 2001).

From an evolutionary point of view, general strategies for optimal defence against predation were formulated, for example that plants are expected to accumulate protective phytochemicals in a tissue or organ in direct proportion to the risk of predation of that unit (Rhoades, 1979). Therefore, organs and tissues with a high nutritional value (seeds,

young leaves, pollen) have a particularly high risk of predation.

Contents

The main secondary metabolites in coffee

The phytochemical catalogue of coffee is very large (for an overview of physiologically active substances see Viani, 1988; Baumann and Seitz, 1992; Clarke and Vitzthum, 2001); however, with respect to chemical ecology, only a few most prominent coffee compounds have been intensely studied so far, namely caffeine, chlorogenic acids and trigonelline. Caffeine is a purine alkaloid (PuA). PuA are divided into methylxanthines (caffeine, theobromine, theophylline etc.) and methylated uric acids (theacrine, liberine etc). 5-Caffeoylquinic acid (5-CQA) is the main component among the coffee's chlorogenic acids. It is a metabolite of the phenylpropanoid pathway and often induced by biotic (pathogens, herbivores) and abiotic (UV,

Table 1: Organism-related effects of caffeine (multifunctionality)

Organism	Effect	Underlying mechanism, target	References
Bacteria and fungi (yeasts)	Bacterio- and fungistatic	Inhibits UV dark repair in DNA)	Kihlman, 1977, McCready et al., 2000
Fungi	Fungistatic, reduces mycotoxin production	Unknown	Buchanan et al., 1981

temperature, nutrient, light) stress. Thus, it is thought to exert specific functions in plant protection such as defending against microbial infection and herbivores, acting like a screen against harmful UV radiation, and scavenging free radicals and other oxidative species (reviewed in Grace and Logan, 2000). As will be outlined below, chlorogenic acid is the ally of caffeine, as it associates with it in a physico-chemical complex (Sondheimer et al., 1961; Horman and Viani, 1972; Martin et al., 1987).

Lastly, trigonelline is a derivative of nicotinic acid and influences many events in plant life (reviewed in Minorsky, 2002): it is a hormone-like regulator of the cell cycle and arrests the cells in the phase prior to mitosis (G2); it has a not yet fully cleared function in nodulation of the legume alfalfa by rhizobial symbionts (interacting individuals of different species); it is believed to be a signal transmitter in the response to oxidative stress; it may act as an osmoregulator; and, finally, it has been reported to induce leaf closure in various species showing sleep movements, the so-called nyctinasty.

Additionally, trigonelline was shown to act as a reserve for the synthesis of coenzymes (NAD)

Plants	Inhibits germination, reduces growth	Not known; inhibits the formation of the cell plate, calcium?	Rizvi et al., 1981, Gimenez-Martin et al., 1968
Mollucs (snails and slugs)	Molluscicidal, reduces heart rate	Calcium release, increases the duration of action-potential plateaus?	Hollingsworth et al., 2002
Insects	Disturbs developmental processes	Inhibits cyclic AMP phosphodiesterase	Nathanson, 1984
Mammals	Activates CNS, constricts cerebral blood vessels, increases lipolysis, positive	Binds to adenosine receptors	Nehlig, 1999

	inotropic		

during early coffee seed germination (Shimizu and Mazzafera, 2000). To our knowledge no report exists regarding the chemical defence properties of trigonelline, though its considerable concentration of around 1% (dry weight) in both seeds and leaves, and even higher in the youngest internode (>2%), this indicating a transport from old to young leaves (Kende, 1960).

Clearly, the chemical defence by caffeine and chlorogenic acids works because of (a) their high concentration in the related coffee organ or tissue, (b) the generally low body weight of the herbivore (a phytophagous insect may be deterred after one cautious trial, while a large naive animal, such as a mammal, may swallow one or several plant organs at a time and thus be intoxicated, but later will avoid that organ and feed selectively on the rest of the plant) and (c) the need of the herbivore to ingest a large quantity of leaves due to their overall low protein content. Very convincingly, the coffee seed, furnished with comparatively high protein has, in addition to the chemical protection, a strong mechanical defence: the endosperm is extremely hard and the inner fruit wall, the endocarp, called 'parchment', is tough.

If we relate chemical defence of the coffee bean to the human being foraging on it, we can state the following: the coffee drinker’s body weight, which nota bene does not result from the protein content of the bean(!), is high compared to the biomass of ground endosperm (roast coffee) used for the coffee brew. And still, some coffee drinkers are only pleased with a plant extract smoothed by sugar, cream or milk. The protecting layers around the bean already have been removed by the lengthy and labour-intense processing of the coffee fruit.

Finally, the target molecule (receptor) of pharmacological action in the coffee drinker is different from that in, for example, an insect (Table 1).

From seed to plantlet

As mentioned in the introduction, biochemical ecology is also a matter of space and time and therefore has to deal with questions such as ‘how and to where is coffee dispersed?’, ‘how long does it take for a seed (= coffee bean) to

germinate?', or 'what happens during the transitions from a seed to a whole plant?'. Most of the results cited below were obtained by studies on arabica but, slightly modified, also apply to robusta.

The hobby gardener may seed a so-called 'green' (unroasted) coffee bean possibly available at the local coffee roasting company. By doing so, he or she has to bear in mind two things. First, the bean rapidly loses its germinating power when harvested and processed, depending on the bean humidity and on the temperature during storage. Generally, within 3 months after harvest and processing, the germination rate drops to zero, but is extended to one full year or longer at 18 °C, when the bean water content never falls below 40%, achieved by storing the beans vacuumpacked in a polyethylene bag (Valio, 1976; Couturon, 1980; Van der Vossen, 1980). Second, under natural conditions the coffee bean will not or very rarely get directly in touch with soil, because the seed-dispersing animals (monkeys, elephants, large birds) attracted by the red, fleshy and sweet coffee berries will, after having ingested them, excrete or regurgitate seeds still covered by the hard endocarp, so to speak a parchment coffee, as it also results technically from wet processing. Occasionally, the entire fruit is dispersed or drops beneath the coffee tree. Then, the berry will dry and shrink very slowly, looking like dry-processed coffee before hulling. The seeds within the shrunk fruit are even better protected and lose less water than in the parchment coffee. In any case, in nature under favourable conditions (humidity) the seed will take up water and germinate, breaking through the outer shell made up either of the parchment or the complete husk. Finally, going back to the hobby gardener, we have to add the following: the naked bean will germinate earlier (by around 2 weeks) than the bean in parch, but has a higher risk of being attacked by pests and pathogens in

Fig. 1 Seed germination and seedling development. (Drawing by Yvonne Boitel-Baur, Zurich, Switzerland). This illustration shows the various stages from the bean in parch up to the emergence of the first foliage leaf pair First row from left to right: Germination, the primary root, and later, the hypocotyl emerge from the seed. Small lateral roots are formed and the hypocotyl extends to carry the 'head' above the ground (c.3 weeks) Middle row from right to left: The apical hook straightens and the cotyledons (not visible) completely invade in and dissolve the endosperm (7 weeks) Lower row left: The cotyledons start unfolding, the remaining unresorbed layer of the endosperm is shed, and subsequently the cotyledons fully expand (10 weeks). The first foliage leaves appear later. In the upper left corner, coffee in parchment (upper row) and 'naked' beans are shown with one of the latter with the

silverskin partially removed

the soil during imbibitions (swelling) and germination. Even though caffeine is described as an effective agent (Table 1) against all kinds of organism (generalists), a few specialists have overcome the defence mechanism and not only detoxify but possibly also metabolize the PuA for their own needs.

Most interestingly, during water uptake (imbibition) caffeine remains almost completely fixed within the bean, very likely due to an efficient caffeine barrier at its surface made out of chlorogenic acids (Dentan, 1985). As will be outlined below, these phenolic compounds are of crucial importance during the entire life of the coffee plant, since they prevent autotoxicity by caffeine! Only when the radicle (the thick primary young root) grows into the substrate, is caffeine released at high concentrations into the environment (Baumann and Gabriel, 1984). Obviously, under natural conditions the imbibed bean is safely encapsulated – there is no need to excrete the defence compound caffeine – whereas during the later stages of germination about one-third of the caffeine of the young seedling, now exposed to pathogens and predators, leaks via the root surface into the substrate, where it may inhibit the competition by other plant species and prepare, along with other compounds, the way for specific root colonization by microorganisms.

As nicely illustrated in Figure 1, the emerging primary root is the first visible sign of germination. However, dramatic changes occur even earlier inside the bean: the tiny cotyledons invade the nutrient-rich storage tissue of the bean, the so-called endosperm, and eventually occupy the entire cavity within the bean. Simultaneously, the hypocotyl (the region of the stem beneath the stalks of the cotyledons) stretches and carries the head above the ground. Finally, the cotyledons unfold and shed the seed coat. Since during invasion into the endosperm they suck up all its constituents, cotyledons are, in simplified terms, a blot of the original coffee bean: they contain and conserve all the caffeine of the endosperm, and hence are most suitable for a screening to detect and select caffeine mutants (Baumann et al., 1998). Similarly, the chlorogenic acids are also transported into the cotyledons where, however, a large fraction is possibly used for lignin synthesis in order to mechanically stabilize the leaf tissue (Aerts and Baumann, 1994). Now the seedling is ready to develop into a plantlet.

From bud to leaf

The leaves are arranged in pairs, which alternate along the stem but are adjusted almost in one plane along the side

branches (see also Figure 2), this in order to optimize light harvest. The individual leaf pair is born from the terminal bud of the stem or of a side branch of first and second order. In the bud, the leaf primordia (tiny pre-formed installations) are covered by a resinous layer followed by two tough scale-like bracts (stipules). The life of the leaf pair starts with its emergence from the bud with the tiny leaf blades still attached to each other. Thereafter, they separate and the individual leaf expands considerably to achieve its final size and shape about 4–5 weeks after emergence. It is still soft, light green and glossy. During the next 2–3 weeks, the leaf texture gets tough and, coincidentally, the upper surface of the lamina turns from glossy to dull dark-green, possibly resulting from a chemical change of the epicuticular waxy coatings: long chain fatty acids are transformed into the corresponding alkanes (Stocker, 1976). Now, 50–60 days after emergence, the leaf is fully developed and optimally gathers solar light energy for the formation of sugars from carbon dioxide and water, or, in other words, the net photosynthesis has reached its maximum where it remains for a long time (Frischknecht et al., 1982; Mösli Waldhauser et al., 1997). Under natural and favourable conditions, the coffee leaf's lifetime lasts for 10–15 months (Van der Vossen and Browning, 1978). Thereafter, senescence starts and finally the leaf is shed.

If we throw a glance on the concomitant course of the key secondary compounds, we find exceptionally high foliar concentrations of PuA and chlorogenic acids as soon as the very tender, nutrient-rich leaflets have left the mechanically protective bud, what can easily be explained by their high risk of predation by, for example, phytophagous insects (Frischknecht et al., 1986). At this stage, both the enzymes (methyltransferases) involved in caffeine biosynthesis and the key enzyme of phenylpropane synthesis, phenylalanine ammonia lyase (PAL), show very high activities (Aerts and Baumann, 1994; Mösli Waldhauser et al., 1997). The leaf alkaloid concentration increases almost to 0.1M (as related to the tissue water) and thus has about 10 times the concentration of an espresso!

The velocity of synthesis of both caffeine and chlorogenic acids decreases sharply during the subsequent leaf expansion. The relative caffeine content drops as a consequence of dilution by growth. However, the absolute amount of caffeine per leaf increases steadily because of low enzyme activities still persisting throughout the entire period of leaf expansion (Mösli Waldhauser et al., 1997). The fully developed coffee leaf has, on a dry weight basis, a caffeine content in the range of the bean.

Amazingly, the chlorogenic acids, even though formed in parallel to caffeine, still continue to increase during the next six weeks (Kappeler, 1988). Shedding leaves have been reported to be caffeine-free, indicating that the caffeine nitrogen is re-used by the plant.

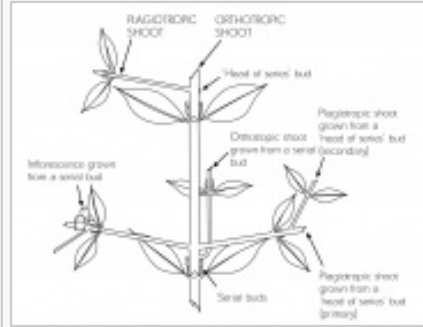

Fig. 2 Dimorphic branching habit of coffee (Cannell, 1985, p. 113)

The concerted formation of both the alkaloids (mainly caffeine) and chlorogenic acids (mainly 5-CQA) has a physiological significance: caffeine easily permeates through all kinds of biological barriers, except a few installed by the caffeine-containing plants themselves (for example, the coffee bean's surface mentioned above). In order to avoid autotoxicity, caffeine is physico-chemically complexed by 5-CQA, and compartmented in the cell vacuole (Mösli Waldhauser and Baumann, 1996). Since chlorogenic acid is encaged in the cells where it was synthesized, one has to assume that caffeine, due to its hydro and lipophilic nature, and to its complexing ability, slowly migrates within the coffee plant towards the sites of highly accumulated chlorogenic acids. In other words, caffeine is passively dislocated and, so to speak, collected within the plant in proportion to the tissue concentration of chlorogenic acid. Apparently, the coffee plant controls caffeine distribution by the allocation of chlorogenic acids. This is nicely illustrated by the uneven distribution of caffeine within the lamina of the coffee leaf: it is highly accumulated at the margins and sharply decreases in concentration towards the mid-rib (Wenger and Baumann, unpublished). Needless to say, the chlorogenic acids show the same distribution pattern. In terms of biochemical ecology it is important to note that this phytochemical leaf architecture is significant: the leaf margin, a preferential site of insect attack, is particularly well furnished with the key defence compounds.

Whether phytochemical leaf architecture is genetically controlled and can be influenced by breeding remains to be

investigated.

Thus, caffeine is an ideal defence compound: the organism feeding on caffeine-containing tissues is unable to hinder it from rapid distribution within and action on its body. However, from the plant's view the problem of autotoxicity had to be solved. The solution was, as already mentioned, complexation by phenolics: all PuA-containing plants allocate high concentrations of either chlorogenic acids (coffee, maté), catechins (cocoa, cola, guaraná), or both (tea). By the use of suspensioncultured coffee cells as a model system, it has been shown that up to 77.4% of the caffeine is fixed in the complex at 25°C (Mösli Waldhauser and Baumann, 1996). Amazingly, in 1964 Sondheimer calculated a complexation degree of 78% for caffeine in the coffee bean at the same temperature (1964). Obviously, the concentration achieved by the remaining fraction (approximately 20–25%) is beyond the autotoxicity level. A temperature increase lowers the degree of complexation and vice versa. In this context, several intriguing questions regarding the interdependence between phenolics and PuA in terms of metabolic regulation, their final concentrations and organ growth rate are not yet fully answered but are well considered (e.g. Ky et al., 1999) in the work on interspecific crosses by the group of Michel Noirot in Montpellier, France (www.coffee-genomics.com).

From flower to fruit

The time period between flower opening (anthesis) and the fully ripe fruit is species-specific and varies considerably among the coffee species. It depends further on the genotype and on climatic and cultural conditions.

The economically important species C. arabica and C. canephora require 6–8 and 9–11 months for maturation, respectively (Guerreiro Filho, 1992).

As might be expected, all flower organs contain the purine alkaloid caffeine, with highest concentrations in the stamens. Amazingly, the latter accumulate, besides traces of theobromine, easily detectable amounts of theophylline, indicating an alternative biosynthetic pathway in the male part of the flower with theophylline as

Fig. 3: Coffee plant (C. arabica) flowering and fruiting. (Drawing by

Beatrice Hasler, Uster, Switzerland). The drawing shows a side branch with flowers at various stages: wilting and falling off, in fresh bloom, or buds (from the base to the apex). Above, flowers are illustrated in detail and enlarged. The yellow stamens are inserted in the throat of the corolla. Similarly, various fruit stages are shown: like the flowers they are arranged in composite clusters at the leaf axil

shown to proceed via theobromine. In analogy to citrus plants, where the highest caffeine concentration has been found in the protein-rich pollen (Kretschmar and Baumann, 1999), we may assume a preferential PuA allocation also to coffee pollen grains. However, the related analyses have not yet been done. Bees are, in contrast to many insects, not only amazingly tolerant against caffeine and other phytochemicals (Detzel and Wink, 1993), but rather, after caffeine uptake, have an improved performance such as a boost in ovipostion by the young queen, an enhanced activity of the bees outside the hive, and an improved defence by bees against hornets at the hive entrance (reviewed in Kretschmar and Baumann, 1999). When the blossom falls from the coffee tree the persisting ovary develops into the young green coffee fruit (Figure 3 and Figure 4).

Fruits always signify high investment costs and, therefore, to defend them against predators the coffee plant pursues several linked strategies.

First, the very young and green fruits are not showy but rather inapparently arranged in clusters in the leaf axil (Figure 3). Secondly, both chlorogenic acids and purine alkaloids are highly concentrated, and, thirdly, the development of the real endosperm is postponed until mechanical protection works. This last point is a most remarkable feature of the coffee fruit development. Within 3–4 months after anthesis the still green fruit reaches a size suggesting readiness for maturation. When cut across, two greenish beans, already typically rolled up, can be recognized.

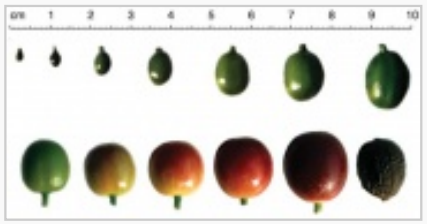

Fig. 4: Fruit development (C. arabica) Above: Within about 4 months the green fruit grows to a

However, the appearances are deceptive: the fruit is far from being mature, the (generally) two beans are false perisperm beans made up of mother tissue (Carvalho et al., 1969). At the adaxial pole (towards the fruit stalk) of each bean one can see the beginnings of endosperm development: a whitish, very soft tissue (also called liquid endosperm) starts to invade into and resorb the perisperm bean.

considerable size. Comparatively late, i.e. between stage 5 and 7, the endosperm starts to develop (2–3 months). Below: When the fruit turns olive, the endosperm is already hard (4–5 months). Now the mesocarp gets fleshy and the exocarp partially red (5–6 months). After 6 months the exocarp is bright red and the mesocarp very fleshy. Later (7–8 months), the fruit colour turns to dark (dull) red and the mesocarp starts to dry out. Finally, the fruit starts to shrink and the exocarp gets dark and darker

Recent studies show that perisperm metabolites such as sugars and organic acids are most likely acquired by the endosperm (Rogers et al., 1999b). We may assume that this process is similar to the invasion of the cotyledons into the endosperm during germination described above: the metabolites shift from one tissue to the other, whereby they have to pass through the so-called apoplast, i.e. the extracellular space between peri and endosperm during seed development, and between endosperm and cotyledons during germination. However, the endosperm is more than a simple blot of the perisperm, since it owns high biosynthetic activities. In conclusion, and philosophically speaking, in coffee the way to the next generation is characterized by transitions in which the metabolites are shuffled around twice.

During this invasion the inner layer (endocarp) of the fruit wall (pericarp) noticeably and increasingly solidifies and later results in the parch layer described above. The mechanical defence of the endosperm itself is remarkably increased by the formation of thick cell walls containing, besides cellulose, the so-called hemicelluloses, i.e. arabinogalactan and galactomannan (Bradbury, 2001).

Hemicelluloses are highly complex polysaccharides primarily renowned for giving an amazing degree of hardness to palm seeds (cf. date, Phoenix dactylifera; vegetable ivory, Phytelephas macrocarpa). It remains to be mentioned that the coffee perisperm finally atrophies into the thin seed coat, the silverskin, that falls off during roasting. Very soon after anthesis the pericarp contains an absolute amount of caffeine kept unchanged until ripeness. However, the initially high (>2%) caffeine concentration drops by dilution to around 0.2% during the further growth and maturation processes, culminating in the transformation of the fruit wall (pericarp) into three distinct layers which serve for fruit dispersal: the tough endocarp protects the seed from digesting enzyme activities in the gut of the frugivores such as birds or

mammals; the fleshy, sugarcontaining (Urbaneja et al., 1996) middle layer (mesocarp) softened by enzymes (Golden et al., 1993) acts as a reward, while the vivid coloration by anthocyanins (Barboza and Ramirez-Martinez, 1991) of the outermost layer (exocarp) is to attract the dispersing animal.

We should not close this section without relating our thoughts about biochemical ecology to a practical question of our daily life: how does the espresso bean get its caffeine? Though numerous publications on caffeine biosynthesis exist (for a comprehensive review see Ashihara and Crozier, 1999), this problem has never been addressed and therefore we can only speculate about it. Clearly, the endosperm has a certain biosynthetic capacity for caffeine. But is this all? Are there contributions of other sources? The perisperm provides around one-third of the seed caffeine as estimated from the caffeine content of the perisperm bean (see Figure 10.5 in Sondahl and Baumann, 2001). The leaves are not directly contributing to seed caffeine, but the pericarp may be a valuable source, as studies with labelled caffeine have shown (Keller et al., 1972; Sondahl and Baumann, 2001).

Obviously, caffeine migrates from the fruit wall into the developing seed, most likely due to the high concentration of chlorogenic acids allocated to the perisperm/endosperm. Unfortunately, the extent of this caffeine transport is unknown. Conceivably, this fraction depends on both the fruit developmental time and the chlorogenic acids allocations, and is correspondingly larger in a slowripening species with a high ratio of seed to pericarp chlorogenic acids.

Again, synthesis, transport and accumulation of chlorogenic acids eventually determine where and how much caffeine is to be allocated in the seed. In conclusion, perisperm and pericarp are certainly important sources of the seed caffeine, whereas the leaves, the pericarp and perhaps also the greenish perisperm may provide most of the chlorogenic acids crucial to gather and firmly fix the caffeine to the coffee bean. However, the degree of contribution from each side (maternal tissues versus endosperm and embryo) is not yet known. Additional studies on the developmental biology of the coffee seed (Marraccini et al., 2001a, 2001b) as well as reciprocal crosses between coffee species differing in their caffeine and chlorogenic acids content will cast some light into the espresso's darkness!

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