W. H.-T. LOH

DNA Plant Technology Corporation, 2611 Branch Pike, Cinnaminson, New Jersey 08077, USA

H. W. MITCHELL

Booker Agriculture International Limited, Masters Court, Church Road, Thame, Oxon OX9 3FA, UK.

J. POISSON

Centre d'Etudes Pharmaceutiques de Châtenay-Malabry, Université de Paris-Sud, Rue Jean-Baptiste Clément, 92290 Châtenay-Malabry, France.

C. J. RODRIGUES, JR

Centro de Investigação des Ferrugens do Cafeeiro, Quinta do Marquês, Oeiras 2780, Portugal

J. SNOECK

Institut de Recherches du Café, du Cacao et autres plantes stimulantes, Avenue du Val de Montferrand, BP 5036, 34032 Montpellier Cedex, France

M. R. SONDAHL

DNA Plant Technology Corporation, 2611 Branch Pike, Cinnaminson, New Jersey 08077, USA

Chapter 1

Genetic Resources of *Coffea*

J. BERTHAUD* and A. CHARRIER*

ORSTOM, Paris, France

1. INTRODUCTION

Coffee trees belong to the botanical genus *Coffea* in the family Rubiaceae. In this chapter, the genetic resources of *Coffea* will be considered. Considerations of its botany have been detailed elsewhere.[1] Here we are more interested in the specificity of genetic resource studies and their utilisation in coffee breeding.

Current commercial green coffee production relies on only two species, *C. arabica* and *C. canephora*, which are described in detail in Chapters 2 and 3. But, in fact, coffee beans can be produced by many other species of *Coffea*. Therefore we do not present data just for these two species; we emphasise the value of all the species. The gene pool useful for *C. arabica* and *C. canephora* breeding is made up of all *Coffea* species, as will be demonstrated later.

In this chapter, we review our present knowledge about wild coffee species. This knowledge has been acquired from studies of wild coffee populations in the forest as well as from experimental hybridisation tests.

New genetic analysis methods have been applied to coffee material. A short description of these methods is given in section 2, and may be useful

* Present address: Centre ORSTOM, 2051 Avenue du Val de Montferrand, BP 5045, 34032 Montpellier Cedex, France.

for an understanding of new coffee breeding methods. Results obtained by these methods lead us to discuss three main points:

(a) Relationships between plants in a population. How are relationships between plants organised in a coffee population? How well adapted are these plants to their environment and to parasites?
(b) Differences between species. What are the main differences between species, from the point of view of ecological adaptation, geographic distribution and genetic organisation? In which species may valuable traits be identified? Can they be transferred from species to species?
(c) Relationships between species. How do hybridisation experiments conducted in recent years implement our knowledge about species differentiation?

As a consequence of this discussion, views are presented on the botanical classification of *Coffea*, on strategies for exploration and conservation of genetic resources, and on interspecific breeding.

2. DISCOVERY OF WILD COFFEE SPECIES, AND ATTEMPTS AT CULTIVATION

2.1. *C. arabica* and Wild Coffee Species

Coffee beverage consumption spread all over Europe during the 17th century, with great profit for the only producing country, the Yemen, where the cultivated species was *Coffea arabica* L. Despite stringent security precautions, some daring Dutchmen succeeded in stealing seeds and in cultivating them in the Dutch colony of Java. Progenies from these first introductions were planted in the Dutch Surinam colony in South America. The Amsterdam botanical garden has been the necessary relay station for this successful introduction. A number of seed robberies established this crop in the French West Indies and in Brazil.[2] This history is summarised in Fig. 1.

Interest in other coffee species came later, during the course of Africa's exploration at the end of the 19th century and the beginning of the 20th century. The discoveries made are now summarised briefly and chronologically.

C. liberica. This species was first discovered in West Africa. Afzelius

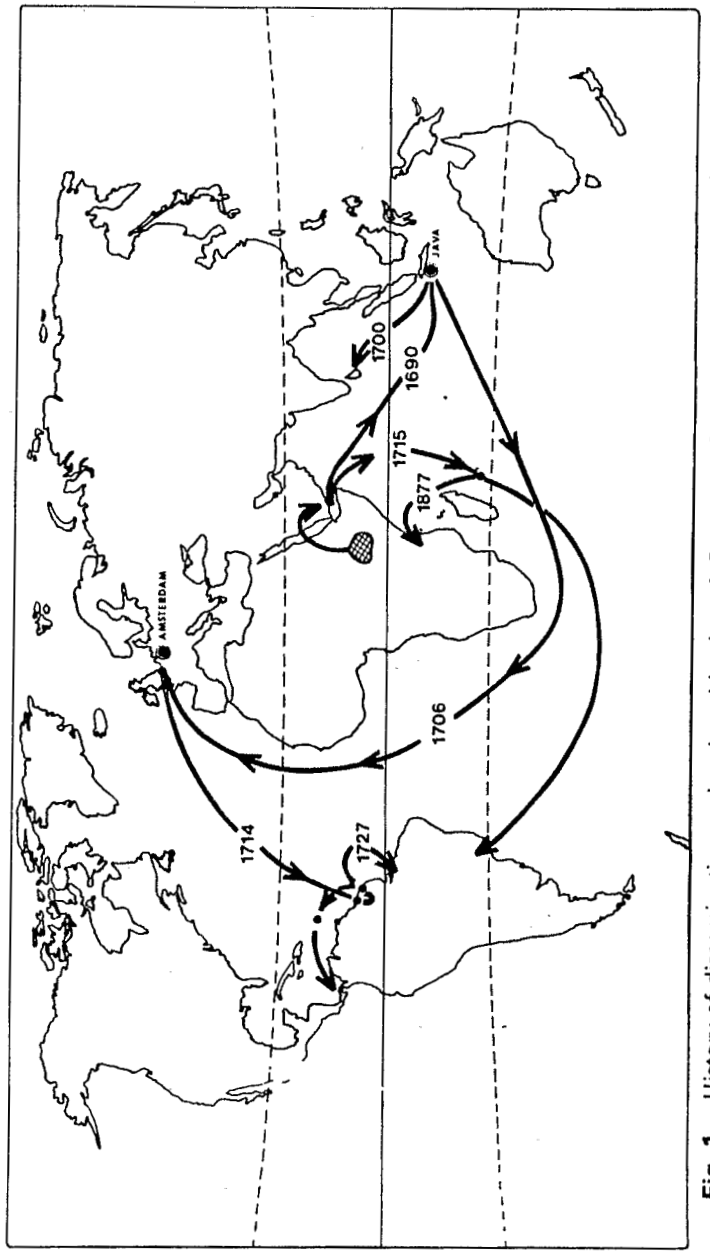


Fig. 1. History of dissemination and early cultivation of *C. arabica*. Cross-hatched area = centre of origin.

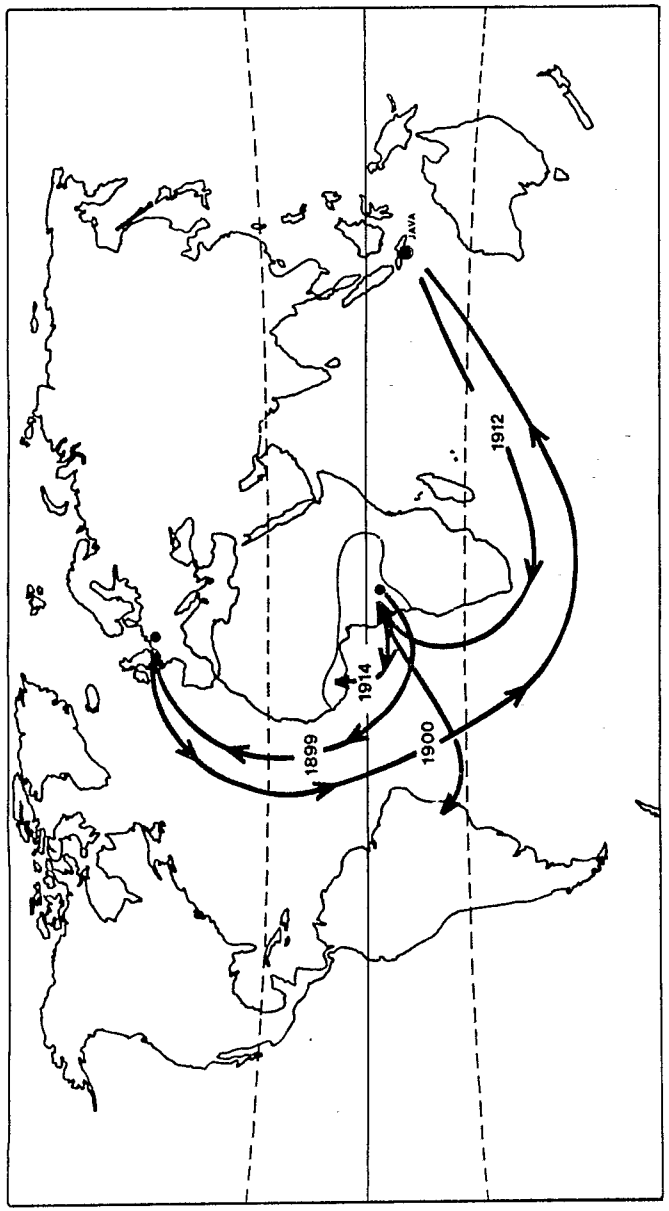


Fig. 2. History of dissemination and early cultivation of *C. canephora*. Cross-hatched area = distribution area of wild populations of this species.

collected samples of cultivated plants in 1792.[2] Extension along the African Atlantic coast took place during the second half of the 19th century. The Central African form was discovered by Chevalier in 1902 during his first travels in Africa.

C. stenophylla. This also was discovered in West Africa, and was first reported by Afzelius in 1794 in Sierra Leone.[2]

C. canephora. This species was cultivated after 1850 on the African Atlantic coast, from south Gabon to north Angola, and especially near the Kouilou river. Independently, it was discovered by Grant in 1861 at Bukoba (Tanzania).[2] The main steps in the extension of the cultivation of this species are shown in Fig. 2. It should be noted that Central African forms were introduced in West Africa (Ivory Coast) at the beginning of this century.

C. congensis. According to Chevalier[2] (citing Sir Harry Johnston) Grenfell, the explorer and missionary, discovered the first *C. congensis* in 1884 in the lower part of the Oubangui river.

The main points in the discovery and attempts at cultivation of coffee species are as follows:

(a) Movements between continents were completed very rapidly, in only a few tens of years. This is true for both *C. arabica* and *C. canephora.*

(b) Cultivation attempts have been made with several species. Only three species were commercially successful: *C. arabica*, *C. canephora* and *C. liberica*. *C. arabica* is well adapted to the highlands, whereas *C. canephora* and *C. liberica* thrive in lowland tropical areas. Tracheomycosis epidemics, caused by *Fusarium xylarioides*, eliminated *C. liberica* species in the field between 1940 and 1950. Now, the world's production is based only on *C. arabica* and *C. canephora.*

(c) Many plant transfers between countries have been achieved, but only a few seeds or seedlings have been involved each time.

The successful production of coffee in the various continents therefore relies on a very narrow genetic basis. In order to broaden the available genetic diversity, new surveys and collections of wild material are fully justified.

Table 1
Coffee surveys led by ORSTOM since 1960. After Charrier and Berthaud,[1] updated

Years	*Countries surveyed*	*Institutions*	*Species collected*	*Location of live collections*
1966	Ethiopia	ORSTOM	*C. arabica*	Ethiopia Cameroon Ivory Coast Madagascar
1960–74	Madagascar area	Museum, IRCC	*Mascarocoffea* >50 taxa	Madagascar
1975	Central Africa	IRCC, ORSTOM	Nana coffee *C. congensis* *C. liberica* *C. canephora*	Central Africa Ivory Coast
1975–81	Ivory Coast	ORSTOM	*C. canephora* *C. humilis* *C. liberica* *C. stenophylla* *Psilanthus* sp.	Ivory Coast
1977	Kenya	IRCC, ORSTOM	*C. arabica* *C. eugenioides* *C. fadenii* *C. zanguebariae*	Kenya Ivory Coast
1982	Tanzania	IRCC, ORSTOM	*C. mufindiensis* *C. zanguebariae* *C.* sp.	Tanzania Ivory Coast
1983	Cameroon	IBPGR,[a] IRCC, ORSTOM	*C. brevipes* *C. canephora* *C. congensis* *C. liberica* *C. staudtii* *C.* sp. *Psilanthus* sp.	Cameroon Ivory Coast
1985	Congo	IBPGR, IRCC, ORSTOM	*C. brevipes* *C. canephora* *C. congensis* *C. liberica* *C.* sp.	Congo Ivory Coast

[a] IBPGR = International Board for Plant Genetic Resources.

2.2. Wild Coffee Collecting

Wild coffee species are only found in Africa and the Madagascar region. *C. arabica* is wild in Ethiopia only. The first collections in this country were made by Cramer during his 1928 visit. During the Second World War, English officers collected a few samples. These first collections permitted the evaluation of the potential diversity existing in Ethiopia. They were a real incentive for new prospecting and collection. One was organised by the Food and Agriculture Organization in 1964,[3] and a second by ORSTOM, France.[4] After these missions, an Ethiopian national programme was set up to organise exploration and conservation of coffee genetic resources in this country.

Wild coffee collecting in Madagascar began during the same period, through a joint initiative of the Paris Museum of Natural History, the Institut de Recherches du Café, du Cacao (IRCC), and ORSTOM (France). After 1975, several surveys and collections were set up in Africa by ORSTOM. The plants collected were established in the Ivory Coast in a genetic resource centre. Currently, living collections in the field have representatives from more than 10 species, and comprise more than 10 000 genotypes.

A summary of these explorations led by ORSTOM in collaboration with other organisations is provided in Table 1 (updated from reference 1). It can be seen that most of the wild coffee areas have been explored, an exception being south-east Africa.

2.3. World Collections

Since many attempts have been made to cultivate new species of coffee, and many exchanges of material have taken place, a strong selective pressure has been applied on this material. Many new genetic combinations have been tested, and useful genes have been maintained. Therefore, coffee plantations are also valuable as a source of genetic material. Exploration within coffee estates is a good way to start a breeding programme, taking advantage of local natural selection.[5] This is especially valuable for *C. canephora* because the gene pool in many countries is built up from several different introductions and local collections. A good example is found in the Ivory Coast. Local exploration and collection can yield good results.

On a world-wide basis, collections are maintained in various coffee-growing countries. These collections are important for conservation of genetic material on a regional basis. Their distribution has already been published.[1] However, a network of genetic resource centres still needs to be organised.

3. TECHNIQUES FOR GENETIC RESOURCE STUDIES

3.1. Surveys and Collections

3.1.1. Surveys

With the purpose of collecting wild coffee trees, the main question one has in mind is: where can wild coffee trees be found?

The first valuable source of information comes from herbarium specimen examination in national and international herbaria. There, 'addresses' for coffee sites can be gathered as well as indications about habitat and variability. A primary survey for strategy and priorities among geographic regions can be set up with this information. A thorough presentation of these problems has been made elsewhere.[6]

Available techniques for rescue determine the type of material to harvest. We took seeds (although generally coffee trees produce few seeds in the wild), seedlings and pieces of hardwood stems. The last were grafted directly on to stocks or allowed to regenerate new softwood stems before grafting.

Conservation of this material is based on a primary living collection in a long- and well-established forest, from which the understorey plants have been removed. Such a collection is located in the Ivory Coast and is divided in two, according to species ecological adaptation. One is at high altitude (1100 m at Mount Tonkoui, Man research station), and the other is at low altitude (Divo, 250 m). However, some species can be maintained only through grafting on to well adapted stocks, as is the case for *C. congensis* grafted on to *C. canephora* in the Ivory Coast. Duplicates of the primary collections are used for specific purposes, e.g. hybridisation experiments and morphological and phenological observations.

3.1.2. Vegetative Propagation

Vegetative propagation is currently used for multiplying selected varieties of *C. canephora*. The technique uses single-node single-leaf cuttings. The rooting of cuttings is a very convenient way to propagate a few genotypes in order to obtain a great number of trees from each one. Grafting is preferred when only a few plants are needed from each genotype. A special value of grafting rests in the fact that almost any portion of the plant can be grafted. Another benefit is the vigour given by the rootstock to the scion. It is therefore possible to rescue weak seedlings and even haploid embryos.[7–9]

In vitro methods can also be used for the same purpose: microcutting and somatic embryogenesis techniques are exploited. For a review, see reference 10 and also Chapter 7 of this volume. However, classical methods remain very much valued.

3.2. Genetic Diversity Evaluation

3.2.1. Chromosome Numbers

Generally, plants (and indeed most living organisms) have two sets of chromosomes, one inherited from the mother and one from the father. Their chromosome number ($2n$) is twice the basic number (x). However, there can also be found polyploid series with plants having a multiple of the basic number: $2n = 3x$, $4x$, etc. In special cases, plants with only one set of chromosomes are encountered: $2n = x$; these are described as haploid.

Evaluation of chromosome numbers is one of the first studies that need to be conducted in the process of genetic evaluation. Plants with different chromosome numbers are difficult to intercross or, at the least, sterility problems may emerge in the offspring.

As a result of chromosome counting, it was observed that almost all coffee species are diploid with $2n = 2x = 2 \times 11 = 22$. The only exception is *C. arabica*, which is tetraploid with $2n = 4x = 44$.

Experimentally, chromosome numbers can be modified. To produce polyploids, the classical and effective method is colchicine treatment. This method was discovered by Blakeslee[11] and used on coffee trees.[12] A routine method was made available by Berthou:[13] colchicine treatment is applied to buds of fast-growing plants and not to seeds or young seedlings.

Haploid coffee plants have been known for a long time, but only in *C. arabica*. They were grouped under the name of variety *monosperma*. In *C. canephora*, haploid embryos rescued through embryo grafting have given haploid plants, from which colchicine-induced diploid plants have been obtained.[8,9]

3.2.2. Incompatibility

Self-compatibility in *C. arabica* has been known for a long time. But it was only in 1959 that a formal proof of self incompatibility—in which a plant rejects its own pollen—was established for *C. canephora*.[14] All the diploid species are in all likelihood self-incompatible.

We have worked out a method for testing compatibility reactions between plants. It is based on observation of pollen growth in excised styles. With this method, incompatibility between different plants can be checked. Using this method, the genetic control of the incompatibility system in *C. canephora* was elucidated.[15] The system is gametophytic with a series of S-alleles. It is identical to the tobacco system, which has been known for a long time.[16]

The method is also useful in estimating S-allele number in wild coffee populations. Such an estimation is valuable in quantifying genetic diversity within populations. Relationships among species are also relevant to this

method, since interspecific incompatibility can be the cause of a low success rate when practising interspecific hybridisation.

3.2.3. Cytogenetic Polymorphism

A. Hybridisation method. The method used is now classic.[17,18] Coffee flowering occurs during the dry season and is triggered by rain showers. The interval between rain and flowering is species dependent. Each blossom lasts only one day, and emasculation and bagging are effected the day before flowering. Emasculation is strictly needed only for *C. arabica*. It has also been found to be very convenient for self-incompatible species since the pollination process is facilitated.

B. Intra- and interspecific combining ability. Obtaining information on intra- or interspecific combining ability between plants is a valuable method for genetic resource evaluation. From an academic point of view, this method provides a better knowledge of species relationships and genetic distances. It is assumed that the easier the two species combine, the closer they are. Information on combining ability may also be of practical value. In order to transfer genes from one species to another, it is necessary to know which barriers are to be overcome. Is there a barrier to the obtaining of hybrids, or are hybrids weakened by sterility problems?

Several parameters are used:

(a) *Cross-success rate*: number of fruits obtained from 100 pollinated flowers. It is also measured by the number of seed or viable seedlings obtained from 100 flowers. These are summary data since a low success rate can be due to different phenomena, such as interspecific incompatibility, poor albumen or embryo development, or lethal genes.

(b) *Male fertility* is estimated from pollen viability. Pollen viability is classically deduced from pollen stainability data. Several techniques are used, e.g. acetocarmine, Alexander, tetrazolium.[19,20]

(c) *Female fertility* is estimated by counts of peaberry (fruits with only one locule) and of empty locules (aborted albumen), by proportion.

(d) *Meiotic behaviour* is based on the following parameters: average number of chromosome associations (univalents, bivalents, etc.); and proportion of pollen mother cells with normal pairing (11 bivalents for a diploid coffee). Meiotic behaviour has an explanatory value, as shown by Louarn[21,22] and Lanaud.[23] A relationship between meiotic regularity and pollen fertility has frequently been detected in hybrid plants.

3.2.4. Progeny Tests

Analysis of the differences between plants from the same family is one way of obtaining an estimation of the genetic diversity of the parents. This tool is more useful with self-compatible species because self-pollinated offspring can be studied and compared with open-pollinated offspring. It has been used with *C. arabica*[24] to get an estimation of residual genetic variation in the wild accessions collected in Ethiopia.[4]

3.2.5. Isozyme Polymorphism

Enzymes are the basic tools of cellular chemistry. Often, an enzyme presents several forms with slightly different structural changes: these different forms are called isozymes. If these forms have different electric charges, they migrate at different speeds when placed in a suitable medium (e.g. starch, polyacrylamide gels) within an electric field. After migration, enzymes are given to their specific substrate. The enzymatic reaction is coupled to a coloured reaction. It is then possible to observe coloured bands corresponding to isozymes. This method offers genetic markers different from the classic morphological markers.

Isozyme analyses can be carried out using different plant tissues. We have used young leaves because they can be removed from old trees in wild populations as well as from young seedlings.

Migration and staining methods are now widely known. Berthou *et al.*[25,26] adapted these methods to coffee characteristics. Currently, seven enzyme systems are used:

Est A and B: esterases *a* and *b*
ACPH: acid phosphatases
ICH: isocitrate dehydrogenases
MDH: malate dehydrogenases
PGD: phosphogluconate dehydrogenases
PGI: phosphoglucose dehydrogenases
PGM: phosphoglucose isomerases

LAP (leucine amino peptidases) and SKDH (shikimate dehydrogenases) have also been used, but interpretation of some of the results was difficult.

Results obtained with this method will be described later.

4. MODE OF LIFE OF COFFEE POPULATIONS

Wild coffee trees live only in the understorey of tropical forests, which are not, however, homogeneous habitats. There is a wealth of different

ecological niches. In this section, we describe how coffee trees are adapted to these habitats, how wild coffee populations are organised, and how genes flow among these populations.

4.1. Habitat

A fine microadaptation of coffee species can be observed while collecting coffee trees.

C. humilis. This coffee species is found along small talwegs made by stream erosion.

C. liberica. In Central Africa, this species is found in gallery forests, but always at the edge, never in the wet part near the stream.

C. stenophylla. In the west Ivory Coast, this species is located on hilltops and not on hillsides or at the bottom of hills.

In other species, localisation may be related to broad edaphic adaptation, e.g. *C. congensis* along river banks, and *C. racemosa* in very dry areas.

4.2. Some Coffee Populations

4.2.1. C. liberica *at Mount Tonkoui (Ivory Coast)*

A coffee population map (Fig. 3) shows heterogeneity in respect of age and location of trees. During propitious periods (for instance after clearing in the forest on account of fallen trees), seed production is heavy and many seedlings develop at the same time from these seeds.

Age groups can be noted that indicate cycles in the occurrence of propitious conditions. Age structure also introduces distortions in reproductive behaviour: at a given time, reproductively active trees can be almost all from the same age group, i.e. originating from only a few parents.

It has been observed that adult trees in the forest produce only a few seeds, generally less than a hundred, even though these same trees can produce thousands of seeds when in a crop field or in a plantation.

Observations on this population were conducted for several years. Only a few trees, as female, are involved in the annual production of seeds, but they differ from one year to another. By progeny testing, we were able to establish that gene flow through pollen is an important factor. A tree with a very small number of flowers does not produce fruits, but can be involved in the next generation as a pollen donor. Pollen transport occurs over long distances (100 m to several kilometres) using insect carriers, and is facilitated by synchronous blossoming of all coffee trees of the same species in a given area.

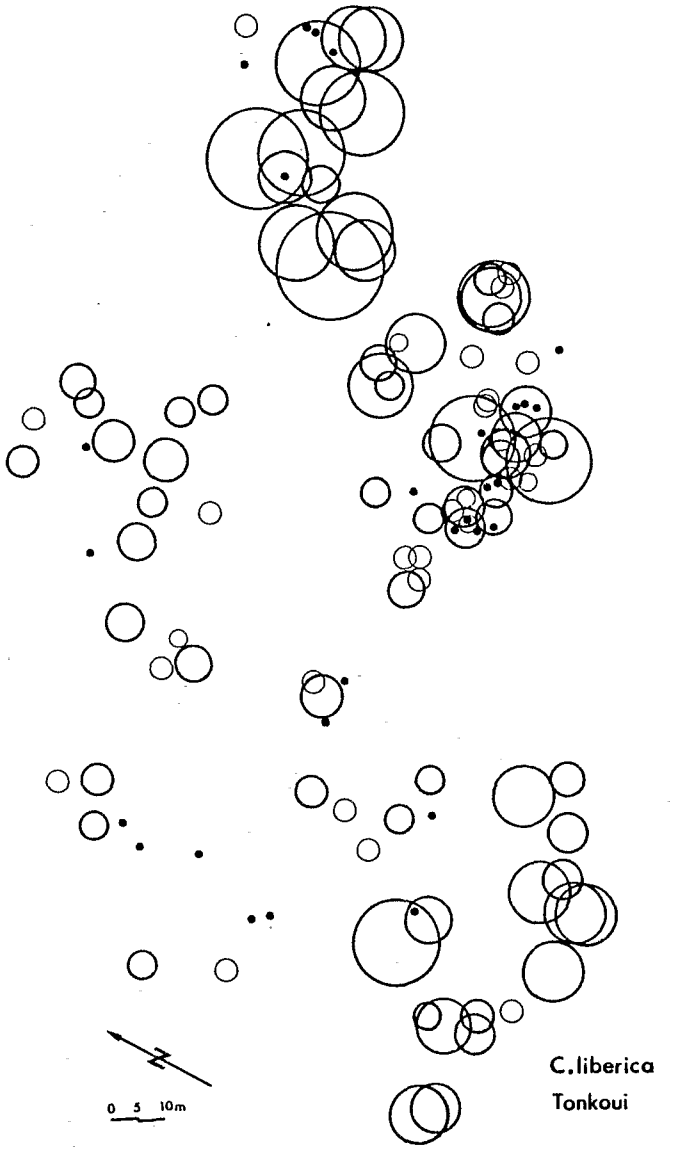


Fig. 3. Map of wild *C. liberica* population at Mount Tonkoui (Ivory Coast). Centre of circle relates to tree position; radius is proportional to tree height.

4.2.2. C. canephora *at Ira Forest Reserve (Ivory Coast)*

Observation of the coffee population map (Fig. 4) reveals a distribution of coffee trees comparable to that of the *C. liberica* population. Clusters of trees of almost identical ages are pointed out. We have then divided the population into three sectors. Genetic markers (isozymes) were used to study this population. With this tool we were able to demonstrate that the oldest tree in the population is a progenitor for only a few trees in this population. When trees are clustered (sector 1 of Fig. 4), they are preferentially pollinated by adjacent trees. When a tree is isolated (02111 in sector 2), it receives pollen from more sources. This can be confirmed by progeny testing. More hybrids with cultivated forms, present in the neighbourhood of this population, are detected in progeny of the isolated tree than in progenies of clustered trees.

Tree position in a population is a strong population gene flow regulatory factor. As a rough approximation, gene flow is proportional to distance; as distance between trees increases, so does gene flow.

There are therefore two overlapping antagonistic trends. When trees are clustered, the trend is in favour of local gene exchange; when trees are more isolated, that trend is towards wide gene exchange. Prevalence of one or the

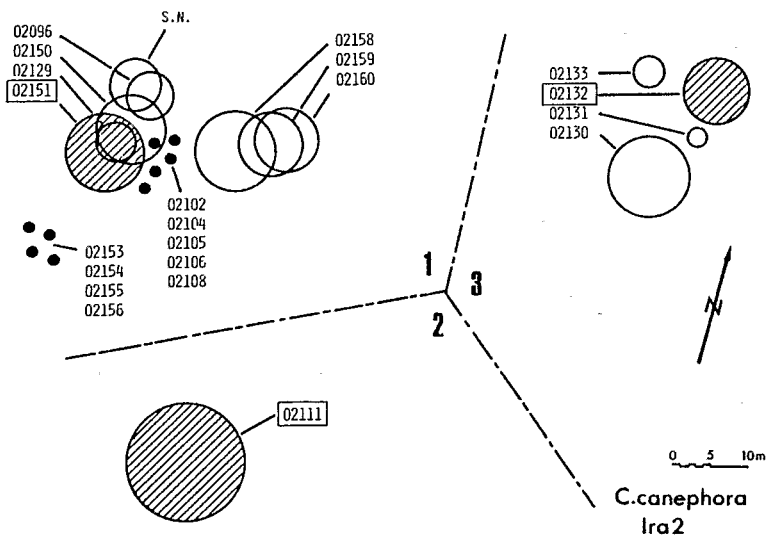


Fig. 4. Map of wild *C. canephora* population in Ira forest reserve (Ivory Coast). Same conventions as in Fig. 3, Cross-hatched circles for trees used for progeny tests.

other factor can explain the existence of homogeneous and heterogeneous populations. As a matter of fact, in the Ivory Coast, all *C. canephora* trees share some enzymatic markers, and this can be taken as proof of common origin or long-distance gene flow. In contrast, a more detailed study in the west central region of the Ivory Coast has shown that typical markers for one region can be identified. In this region, we have also discovered a population with most of its elements resistant to rust (*Hemileia vastatrix*) when all other populations in the same area—a few kilometres away—were sensitive or very sensitive.

4.3. A Disease in a Coffee Population

For several years we were able to study a disease in wild coffee populations. This study was conducted in a *C. humilis* population for rust reactions. All *C. humilis* trees are very sensitive to both rust species *H. vastatrix* and *H. coffeicola*, as shown in living collections.

In the wild population, infestation varied from one year to the next and was never serious. Ten per cent of plants were infected by *H. vastatrix* in one year, 1976, and 5% by *H. coffeicola* in another year, 1981. Between these two years, the infestation level was lower. During a five-year period, 25% of the tree population were infected, and never in an epidemic way. This result proves that the host–parasite relationship is very different in a natural ecosystem from that in an agrosystem.

Knowledge of disease–plant behaviour in a natural ecosystem cannot be used for predicting disease behaviour in crop field conditions. An important consequence for genetic resource collecting strategy may be drawn. Evaluation for disease or pest resistance should be carried out only in crop field conditions. In the wild, in the forest, an absence of damage on some plants does not mean that these plants are more resistant than their neighbours.

5. SPECIES ORGANISATION

In this section, we explore species organisation diversity, pointing out differences in distribution areas and genetic structure.

5.1. Distribution Areas

The distribution area of coffee trees is linked to the distribution area of tropical forest, at least in West and Central Africa. In East Africa, a

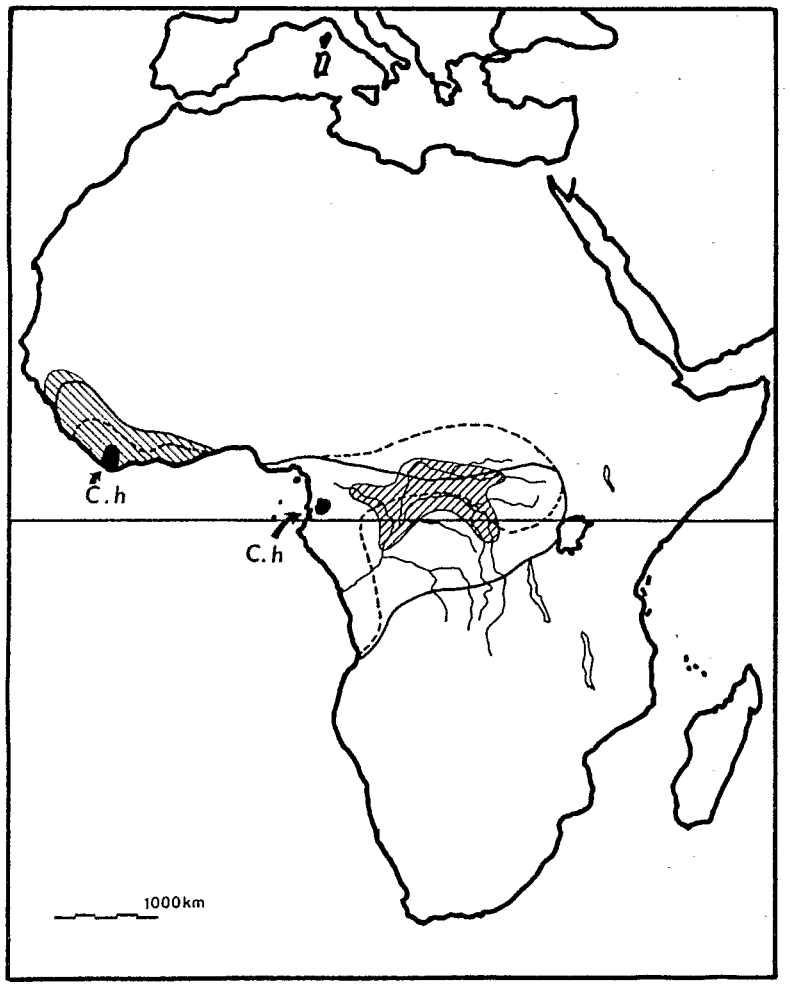


Fig. 5. Distribution area of some coffee species.

different pattern is observed, where tropical forest is not present in solid blocks but is a particular stage of vegetation in a series of vegetation stages found on the slopes of mountains. Therefore, forest and coffee distribution areas in this region are patchwork-like.

Species differ greatly in the size of their distribution areas, as can be inferred from the map (Fig. 5). Some species have a very restricted area, i.e. they are endemic, which is the case for *C. humilis* in West Africa, and *C. fadenii* and *C. mongensis* in East Africa. In the central Atlantic region, many species are not well described and have small distribution areas which still need to be better defined.

Other species are more widely distributed, but do not fully cover a forest block. *C. congensis* is an example of this situation. Another example is *C. arabica*, found only in south-east Ethiopia and on the Boma Plateau in Sudan. Mount Imatong (Sudan) and Mount Marsabit (Kenya) are also refuges for small populations of this species. *C. stenophylla* is found in the West African forest block but is absent from the Central African forest block. *C. zanguebariae* has an almost linear distribution as it lives only in coastal forest in East Africa. It is found along an extensive range: from the Kenya–Somalia border to the Mozambique–South African border. *C. canephora* and *C. liberica* are two species with wide distribution. Their distribution area is almost identical to the tropical forest area.

In Table 2 we give a summary of the distribution areas of particular species, broken down by regions.

Table 2
Distribution of *Coffea* species by geographic region

West Africa	*Central Africa (Atlantic)*	*Central Africa*	*East (and south-east) Africa*
C. canephora	*C. canephora*	*C. canephora*	*C. fadenii*
C. liberica	*C. liberica*	*C. liberica*	*C. mongensis*
C. humilis	*C. humilis*		*C. mufindiensis*
C. stenophylla	*C. congensis*	*C. congensis*	(complex of)
	C. brevipes	*C. eugenioides*	*C. racemosa*
	C. staudtii		*C. salvatrix*
	C. sp. C30		*C. zanguebariae*
	C. sp. C34		(complex of)
		C. arabica (Ethiopia)	*C. rhamnifolia (Baracoffea)*
$n=4$	$n=8$	$n=4+1$	$n=6+1$

Explanations for these differences are not simple, and are perhaps out of place in this chapter. However, two factors should be mentioned:

(a) range of ecological adaptations of these species;
(b) history of species dispersion and events related to tropical history as a whole.

5.2. Genetic Structures within Species

After this description of species distribution patterns, studies can proceed further at the genetic level, to provide a better understanding of the relationship between populations of the same species. Most of the information on this subject comes from isozyme analyses.

5.2.1. Monomorphic Species

From the study of its isozymes, *C. arabica* can be seen as a monomorphic species. As a matter of fact, only one isozyme pattern has been seen, with the exception of an acid phosphatase variant in one population.[25]

Within *C. congensis* forms of Central Africa (the only ones analysed), no discontinuity has been observed. All the populations have a common range of diversity, which has been estimated through isozyme techniques[26] and morphological and phenological observations.[27]

C. humilis is also a monomorphic species, judged at least on the basis of the analysed material,[26] which was collected only in the Ivory Coast. However, a strong intrapopulation diversity has been found, even though this species has endemic characteristics.

5.2.2. Polymorphic Species

C. stenophylla. Isozyme analysis of the Ivory Coast populations of this species showed an organisation in two clear-cut groups. Each group is homogeneous; only one allele is found for each enzyme gene. At each locus a different allele is found in these two groups.

C. zanguebariae. Previous herbarium observations have shown the existence of distinct forms, the length of flower stalk being the most conspicuous distinctive character. In her revision of the genus *Coffea* in East Africa, Bridson[28] has described several species based on these forms: *C. pseudozanguebariae* and *Coffea* sp. A for the northern area, and *C. zanguebariae* in the south. After our collection in Kenya, Hamon *et al.*[29] carried out isozyme and morphological studies on this material and concluded that two forms really existed. We named these two forms A and

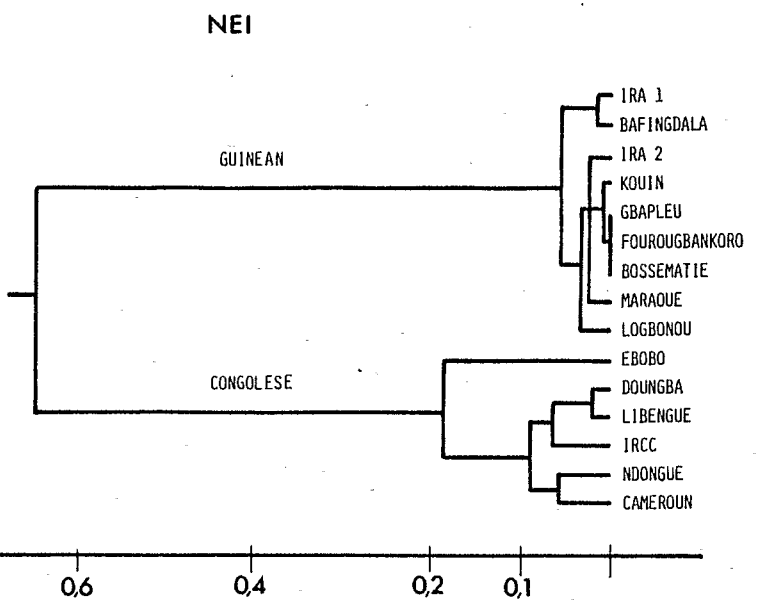


Fig. 6. Genetic organisation of *C. canephora* species based on Nei's genetic distance. Dendrogram was established using genetic distances between populations distributed over the distribution area.

B.[30]* They are easily distinguished by morphological characters, and their isozyme patterns are different. Genetic isolation relies on non-overlapping flowering and hybridisation barriers. However, a low proportion of intermediate (hybrid) forms have been found in populations where both typical forms coexist.

C. canephora. This species was thoroughly studied using material collected in the wild or formerly established in Ivory Coast collections. Two groups were pointed out (Fig. 6). One includes West African forms and we named this 'Guinean'; the second, 'Congolese', includes Central African forms.[30] The geographic limit within these groups is the gap between West and Central African forest blocks. The consequences of this genetic structure for the breeding of *C. canephora* will be detailed in Chapter 5 of this volume. New collections are necessary for an in-depth description of the genetic structure of coffee of the central Atlantic zone.

* After this chapter was completed, we read a paper by Bridson describing *Coffea* sp. A under the complete name *Coffea sessiliflora.* Then, considering taxonomic units, we have equivalence between *Coffea sessiliflora* Bridson and *C. zanguebariae* form A (reference: Bridson, D. M. (1986) *Kew Bull.*, **41**, 307–11).

C. liberica. This species shares with *C. canephora* an identical distribution area and an identical genetic organisation. Forms from the West African and Central African forest blocks are different. This separation has been shown through isozyme analyses[26] and through morphological and agronomic characters. Small leaves and large fruits accompany West African forms, whereas large leaves and small fruits are typical of Central African forms. Several different species have been described to account for this variation: *C. liberica* in West Africa, *C. dewevreii* in Central Africa, along with *C. dybowskii*, *C. arnoldiana*, *C. excelsa* and others.

It is clear that both species share an identical distribution area and genetic structure. However, at the border between groups, especially in the central Atlantic zone, relationships remain poorly understood, mainly because of poor collecting efforts in this region.

In Table 3, we have reported typical situations, comparing distribution areas, genetic structure, and average genetic diversity (measured by average number of alleles per population or per species, ANA P or S). The purpose was to show that genetic structure is not always just superimposed on distribution area structure.

Table 3

Distribution areas and genetic structure characteristics for some *Coffea* species

Species	*Distribution*	*Genetic structure*	*ANA Pop*	*ANA Sp*
C. canephora	West and central African forest blocks	G C	1·7	3·5
C. liberica		G C	1·8[a]	3·1[a]
C. stenophylla	West African forest block	WIC EIC	1·1[a]	2·0[a]
C. humilis	Liberia Ivory Coast		2.4[a]	3·1[a]
C. congensis	Zaire basin		1·7[a]	1·9[a]

G: Guinean group; C: Congolese Group; WIC: West Ivory Coast form; EIC: East Ivory Coast form; ANA: Average number of alleles per locus per population/per species.

[a] Estimated from data published by Berthou *et al.*[26]

Within *C. stenophylla*, two well defined genetic groups are found while this species is located in only one forest block. Within *C. zanguebariae*, genetically distinct forms are found in the same population.

The actual genetic differentiation within a species is a particularly complex phenomenon linked to isolation. But isolation can proceed from genetic barriers based on various mechanisms or from geographic distance. Current genetic differentiation can reflect previous geographic isolation as all these species have experienced a complex history.

We can draw the following conclusions on the future collecting of wild coffee populations. For each species, it is particularly important to explore the whole distribution area, even if a large-scale sampling scheme is used. This first approach should give an idea of specific genetic structure and provide guidelines for continuing species exploration.

Genetic structure analysis within species should be conducted concurrently with, or before, interspecific genetic-relationship studies. It is one way to introduce realistic samples of each species in interspecific studies.

6. RELATIONSHIPS AMONG SPECIES

6.1. Multispecies Populations

The previous description of distribution areas and ecological adaptations can lead to the idea that different species are found in different places. As a result of our survey missions, we can report the existence of multispecies populations. We observed the following associations:

C. liberica + *C. canephora* (Gbapleu, Ivory Coast)
C. liberica + *C. humilis* (Taï, Ivory Coast)
C. liberica + *C. stenophylla* + *C. canephora* (Ira, Ivory Coast)
C. liberica + *C. congensis* + *C. canephora* (Bangui, Central Africa)

In Madagascar, several associations have also been observed. In Uganda, Thomas[31] reported the existence of *C. canephora* and *C. liberica* or *C. eugenioides* multispecies populations.

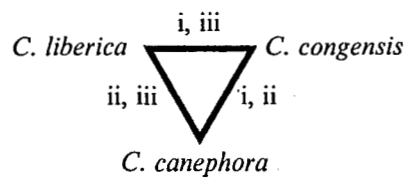
These observations allow us to point out some facts.

First, interspecific hybridisation in wild populations is a phenomenon that is seldom actually seen. In the Ivory Coast, we have never found hybrid plants in multispecies populations, but we have been able to detect hybrid forms in seedlings from seeds harvested in these populations. Interspecific hybrids relate to a transitory stage. Thus, the chance of observing these hybrids in natural conditions is very low.

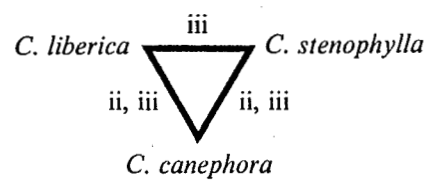
Several factors limiting gene exchange within multispecies populations can be established. We have already proposed three possible mechanisms actually observed to be at work in Central Africa.[32]

(i) Different localisations: *C. liberica* and *C. canephora* may coexist, but *C. congensis* never does so with one of the former species.
(ii) Shift in the timing of flowering: maximum blossom period for *C. canephora* occurs sooner than for *C. congensis* and *C. liberica.*
(iii) Different latent periods between a triggering rain shower and flowering. Anthesis for *C. liberica* occurs 6 days after being triggered by rain, whereas 7 days are necessary for *C. canephora* and *C. congensis.*

Within these three species, working isolating mechanisms are distributed as follows:



In the Ivory Coast, in the Ira forest reserve multispecies population, some of these mechanisms are found for *C. canephora*, *C. liberica*, and *C. stenophylla.*



The effectiveness of the reported isolation mechanisms restricts gene flow between different species, but does not preclude all opportunities for gene exchange. Therefore, gene exchanges between coffee species in multispecies populations are currently active.

6.2. Experimental Interspecific Hybridisation

Results from these experiments help to characterise cytogenetic relationships between species and to identify reproductive barriers between species.

6.2.1. Relationships among Diploid Species

A report of extensive work done in Brazil has been published by Carvalho and Monaco[33] (see also Chapter 4). Since then, with material collected in wild populations, Louarn[21,22,34,36] has completed a large-scale experimental hybridisation programme. This work can be seen as a continuation of a programme initiated in Madagascar with the wild coffee of that country.[35] Here, we present results based on parameters such as cross-success rate (Fig. 7) and cytogenetic behaviour for some diploid combinations (Table 4).

Hybrids between most of the species have been produced. Missing combinations failed mainly because no good specimens were available for these species. Louarn[34] classed combinations in three categories according to their cross-success: (i) more than 19 hybrids produced for 100 pollinated flowers; (ii) 5 to 18; (iii), less than 5. When the results are tabulated by species, it is observed that the best results are found when *C. eugenioides* or *C. congensis* is involved in the combinations. With *C. canephora*, results are clearly lower. Thus, these results can be used to give an estimate of 'interspecific general combining ability'.

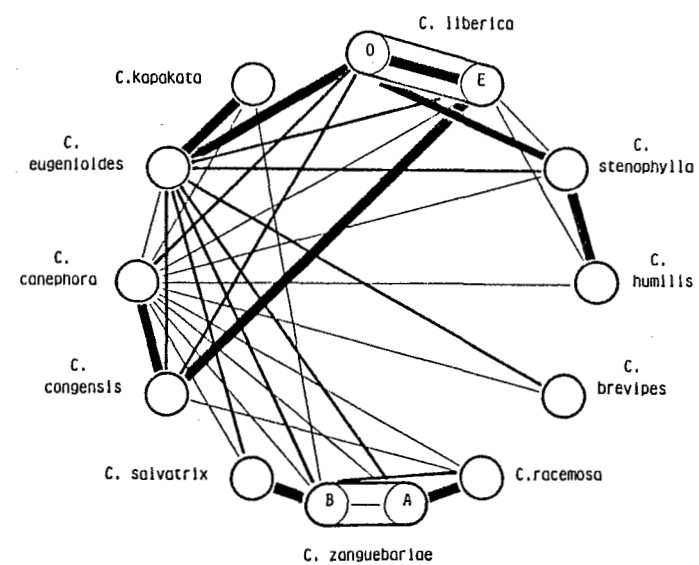


Fig. 7. Cross-success rate in diploid *Coffea* interspecific hybridisation. Adapted from Louarn.[34] Wide line: more than 19 hybrids per 100 pollinated flowers; intermediate line: between 5 and 18; thin line: less than 5.

Table 4
Review of the cytogenetic behaviour of interspecific crosses between diploid *Coffea* species

Diploid F_1 hybrids	*Chromosome associations*		*PMC with 11 bivalents (%)*	*Pollen fertility*	*Reference*
	Univalents	*Bivalents*			
C. canephora × *C. congensis*	0·04–0·74	10·63–10·98	—	high	Leliveld[62]
	0·20–0·52	10·74–10·90	74–90	89–93	Charrier[35]
	normal meiosis		—	<50–>90	Berthaud (unpublished)
C. canephora × *C. liberica*	1·44	10·28		middle	Leliveld[62]
	0·30–1·40	9·93–10·66		39	Chinnappa[63]
	1.16–1·20	10·40–10·42	50–58	64	Louarn (unpublished)
C. canephora × *C. eugenioides*	1·30–2·22	9·89–10·35	23–44	43	Louarn[21]
C. liberica × *C. eugenioides*	about 2 univalents				Vishveshwara[64]
	1·28–1·64	10·18–10·36	42–48	35	Louarn (unpublished)
C. canephora × *C. racemosa*	1·5–6·0	8–10	0–45	0–8	Louarn[36]
C. canephora × *C. kapakata*	1·50	10·25		middle	Leliveld[62]
C. perrieri × *C. kapakata*	3·20–3·40	9·30–9·40	7–23	6–8	Louarn (unpublished)
C. perrieri × *C. salvatrix*	0·44–3·26	9·37–10·78	10–78	7–35	Louarn (unpublished)
C. perrieri × *C. zanguebariae*	3·12–3·32	9·34–9·44	12–14	13–?	Louarn (unpublished)
C. racemosa × *C. perrieri*	0·72–1·12	10·44–10·64	56–70	5–24	Louarn (unpublished)
C. canephora × *C. lancifolia*	3·20	9·40	8	8	
C. canephora × *C. resinosa*	4·40–6·40	7·80–8·80	0–2	4–7	Charrier[35]
C. canephora × *C.* sp. A311	5·04	8·48	4	6	
C. perrieri × *C. eugenioides*	2·94	9·34	20	1	Louarn (unpublished)
C. perrieri × *C. liberica*	5.13	8·43	7	1	Louarn (unpublished)

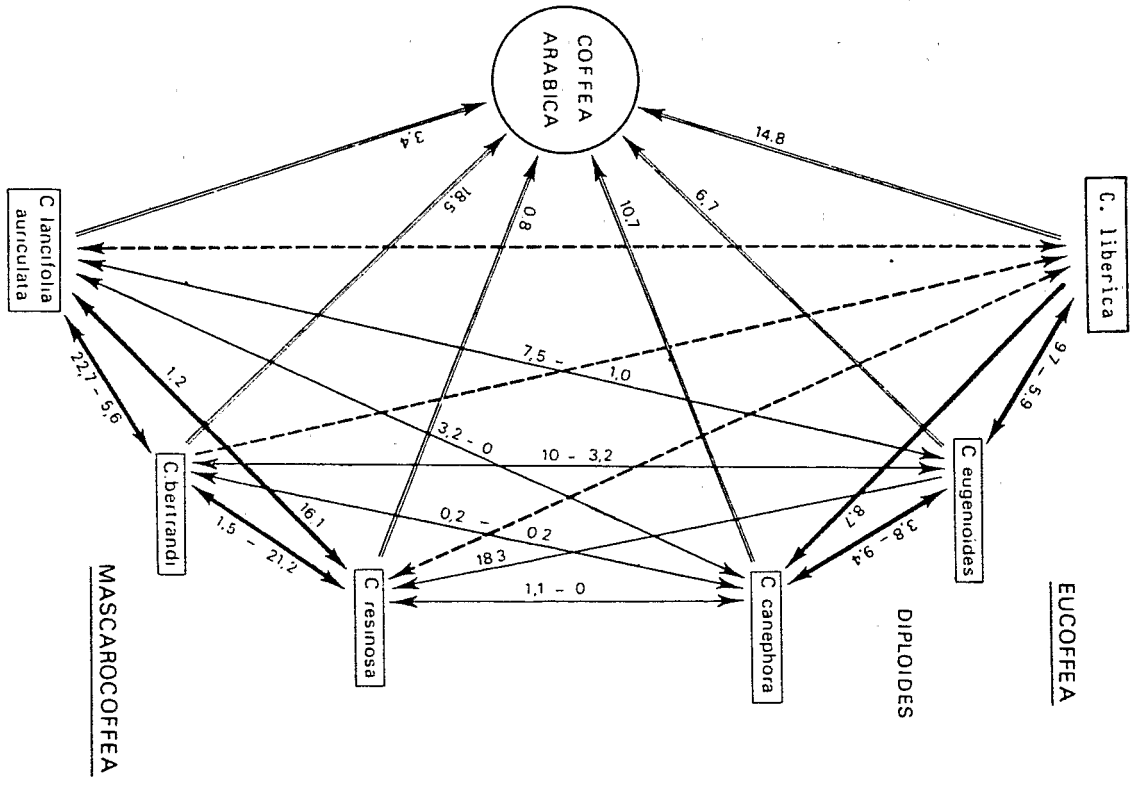


Fig. 8. Cross-success rate in *C. arabica* × diploid *Coffea* species hybridisation. After Charrier.[38]

Table 5
Review of the cytogenetic behaviour of autotriploids of *C. canephora* and interspecific crosses between *C. arabica* and diploid *Coffea* species

Triploid F_1 hybrids	*Sample size*	*Chromosome associations*				*Reference*
		I	*II*	*III*	*Others*	
C. arabica × *C. canephora*	1	14·4	5·4	2·60	—	Krug and Mendes[65]
	1	7·8	9·75	1·61	0·21	Kammacher and Capot[66]
	1	7·98	9·5	1·93	0·04	Chinnappa[67]
	1	9·87	9·57	1·33	—	Louarn (unpublished)
C. arabica × *C. congensis*	5	9·2	8·6	2·2	—	Louarn (unpublished)
C. arabica × *C. eugenioides*	9	9·5	9·3	1·7	—	Louarn (unpublished)
C. arabica × *C. kapakata*	1	10·07	9·45	1·33	—	Monaco and Medina[68]
C. arabica × *C. liberica*	2	9·28	9·64	1·44	0·03	Charrier[35]
	1	10·9	9·5	1·0	0·04	Louarn (unpublished)
C. arabica × *C. stenophylla*	2	10·7	9·6	1·1	—	Louarn (unpublished)
C. racemosa × *C. arabica*	1	11·3	9·7	0·80	—	Medina[69]
C. arabica × *C. racemosa*	2	11·2	9·6	0·9	—	Louarn (unpublished)
C. arabica × *C. bertrandii*	1	11·9	8·6	1·3	—	Charrier[35]
C. arabica × *C. perrieri*	1	12·4	8·2	1·4	—	
C. arabica × *C. pervilleana*	1	14·3	8·6	0·5	—	
C. arabica × *C.* sp. A311	1	17·1	7·7	0·2	—	
Autotriploid *C. canephora*	2	2·8	2·8	8·2	—	Louarn (unpublished)
	1	2·75	3·46	7·25	0·38	Sreenivasan[70]

Fertility of interspecific hybrids varies widely and relies on several mechanisms.[36] In the *C. canephora* × *C. congensis* combination, hybrids have a regular or almost regular meiotic behaviour, but some of them have a low pollen fertility. Disparate rates of pollen fertility have been observed among individuals resulting from a single cross (same parents). In this case, low fertility is determined by the presence of special genes.

In other combinations, such as *C. canephora* × *C. racemosa*[36] or *C. canephora* × *Mascarocoffea* (Table 4 bottom), meiosis is very irregular and pollen fertility very low. Thus, sterility is a consequence of chromosomal differentiation.

Therefore, with such disparate results, it is very difficult to estimate 'distances' between species. It is necessary to test several genotypes within each species to obtain reproducible results and a clear picture of relationships among species.

Advantage can be taken of this behavioural diversity. Even when hybrids between two species are produced with difficulty, choice of special genotypes should permit the obtaining of hybrid individuals.

6.2.2. Relationships between Diploid Species and C. arabica Tetraploid

Many triploid combinations have been tested. Diploid species in these combinations are of African or Madagascar origin. The results reported in Fig. 8 and Table 5 are adapted from Charrier[35] and updated with Louarn's data (J. Louarn, personal communication).

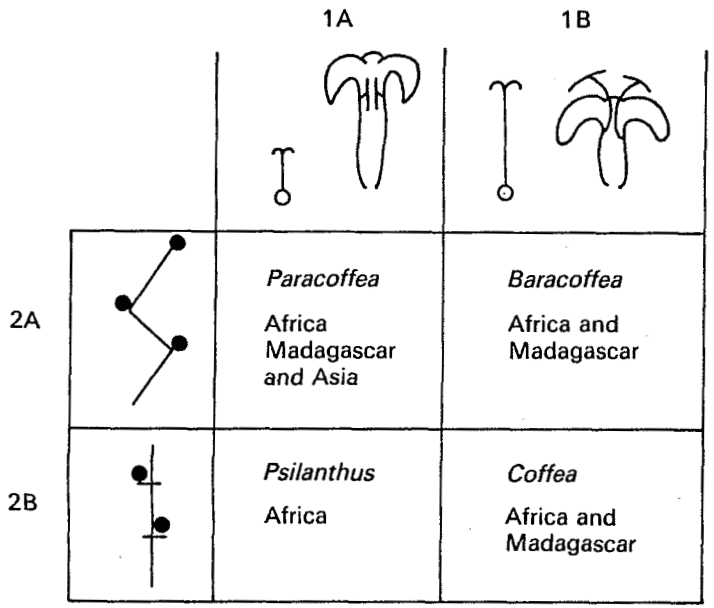
Triploid hybrids were obtained with many species and with good cross-success (an average of eight hybrids for 100 pollinated flowers), though they are all sterile. Meiotic behaviour is reviewed in Table 5. All species share a common behaviour: a configuration typically observed is 11 univalents + 11 bivalents. Within the haploid genome of *C. arabica*, pairing is limited: an average of 4·5 bivalents out of 11 potential bivalents is observed.[37,38] Therefore, as already stated by Charrier,[35] all coffee diploid species share a common base genome; this genome is found in *C. arabica* also.

7. EVOLUTION AND TAXONOMY OF *COFFEA*

7.1. *Coffea* Genus Characteristics

In this section we will not discuss the formal taxonomy of the genus *Coffea* as this has already been described elsewhere.[1] We will instead point out the consequences of the previously described situations, and postulate some

Table 6
Keys for *Coffea* intra-genus classification. Adapted from Leroy[39]



1A: Long corolla tube, anthers not exserted, short style.
1B: Short corolla tube, anthers exserted, long style.
2A: Terminal flowers, predominantly sympodial development.
2B: Axillary flowers, monopodial development.

interpretations. One of these will include a proposal for classification within the genus *Coffea*.

Leroy[39,40] differentiates the genus *Coffea* from other genera within the Rubiaceae family on the basis of gynoecium and placenta types. This author has proposed four subgenera, which distinction is based upon two main criteria: flower shape and growth habit (Table 6). In this review, we consider only *Coffea*, excluding *Baracoffea*, *Paracoffea* and *Psilanthus*. This subgenus contains species placed by Chevalier[41] in both the *Mascarocoffea* and *Eucoffea* sections.

The main points considered in the preceding sections and to be discussed are as follows:

(a) Distribution areas are very diverse. Some species are widely distributed whereas others are found only in very restricted zones.

(b) The number of species is unevenly distributed throughout the regions.
(c) Species are not always homogeneous. Group structures can be found.
(d) Cytological studies show only one basic genome for all diploid species, with a slight genomic differentiation for coffees from Madagascar and East Africa.
(e) Interspecific hybridisation barriers do not 'coincide' with species limits defined by taxonomists.

How original is this *Coffea* organisation? Does it follow a common scheme also found in other plants and in flora in general?

7.2. Organisation of Tropical African Flora and Fauna

A study of the African tropical flora, or at least of some species and families, reveals the existence of botanical regions on this continent, based on richness in endemic species and abundance of species. White[42] described three regions or centres which we have named A, B and C. Centre A is in the Guinean forest block; B and C are in the Congolese forest block. Diversity found in these centres can be summarised as follows for 277 species studied:

110 were found in A, including 30% of endemic species
210 were found in B, including 34% of endemic species
146 were found in C, including 23% of endemic species
243 were found in A + B, including 43% of endemic species
244 were found in B + C, including 43% of endemic species

Centre B, the central Atlantic centre, is richer on both a species-number and an endemic-species-number basis. Between these three centres, two dividing zones, or intervals, are found. One is between West and Central Africa. At the present time there is no forest in this area. The other one, the Sangha interval, between B and C, is located in a vegetation zone not different from B or C.

Other botanists[43,44] have demonstrated that the forests of Kenya and Ethiopia are floristically linked with the Central African forest. On the other hand, the Indian Ocean coast zones are very different. They share homology only at the genus level, not at the species level.[45] In this coastal region, a hot spot for species diversity and endemism is in Mount Usambara (Tanzania) and surrounding hills.

This general scheme for specific diversity distribution is also found for animals (see reference 46 for a review and references 47 and 48 for studies on birds).

Therefore, a common trend is found in Africa for species distribution. Coffee species distribution follows this general pattern. According to Table 3, more species are concentrated in the central Atlantic region than in other regions. East Africa is also rich in endemic coffee species. Some coffee species are restricted to the top of one or a few hills, such as *C. fadenii* or *C. mongensis*.

7.3. Structuring Forces

Since coffee trees and the flora as a whole are similarly organised, common causes for this organisation may be looked for. Several authors[46,49] have proposed a 'refuge' theory. Over geological periods, forest distribution in Africa has not been stable. Phases of retreat and expansion have alternated. During expansion phases, recolonisation has begun from especially propitious areas, or 'forest refuges', where forest was able to survive during unfavourable periods. Currently, in these particular areas or 'refuges', the largest species diversity should be found.

These forest fluctuations would be induced by climatic variations. During the Quaternary period, several glaciations were experienced. Ice sheets covered part of all continents, and in tropical latitude regions cold and dry weather was the rule. These weather modifications led to forest retreat and losses of species or at least of diversity within species. During deglaciation phases, forest would have recolonised the same areas very quickly. Climatic variations and forest area oscillations would have been forces structuring African flora and fauna diversity. Coffee trees should be a good example of this genetic structure.

Based upon coffee distribution at the present time and refuge area locations, a map of coffee distribution for the last glacial maximum (18 000 years ago) has tentatively been drawn (Fig. 9). From refuge areas coffee would have recolonised the zones they occupy now, according to the routes proposed in the map in Fig. 10. This map could represent the actual genetic diversity distribution within and among coffee species.

7.4. *Coffea* Evolution

Observed flora and fauna divergences between Central Africa and the East African coast are also found in the genus *Coffea*, and groups may be delimited. We named the West and Central African group *Erythrocoffea*, and the East African *Mozambicoffea*. This latter group shares numerous characteristics with *Mascarocoffea* (*Coffea* from Madagascar).

This leads to the idea of three existing sections within *Coffea*: two African

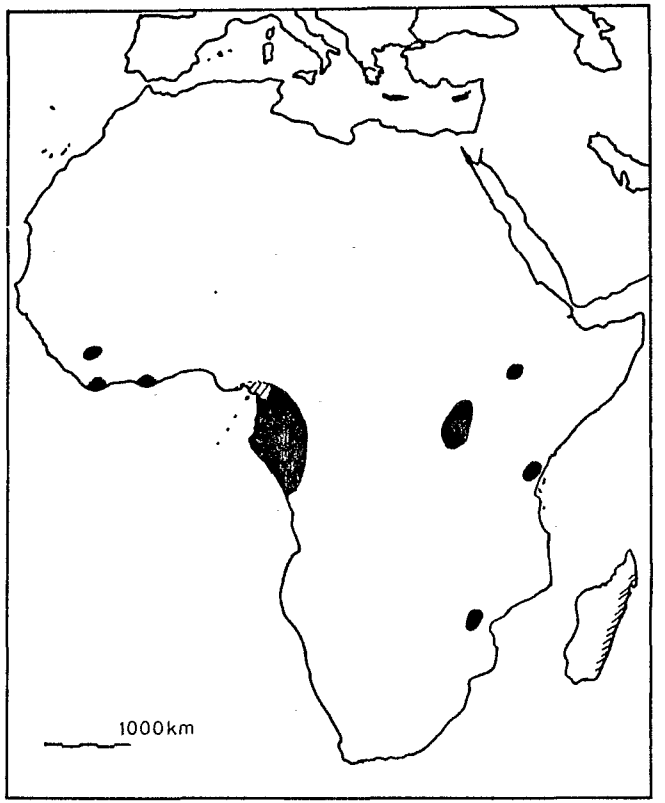
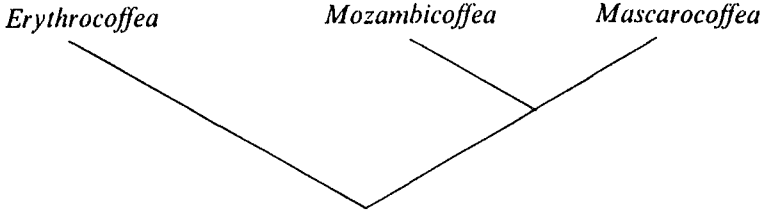


Fig. 9. 'Refuge' zones for *Coffea* species at 18 000 years ago. After Hamilton[46] and personal interpretations.

ones, and one from Madagascar. Phylogenetic relationships between these sections can be represented as follows:



This classification is not based on morphological characters only, as was the one of Chevalier.[41]

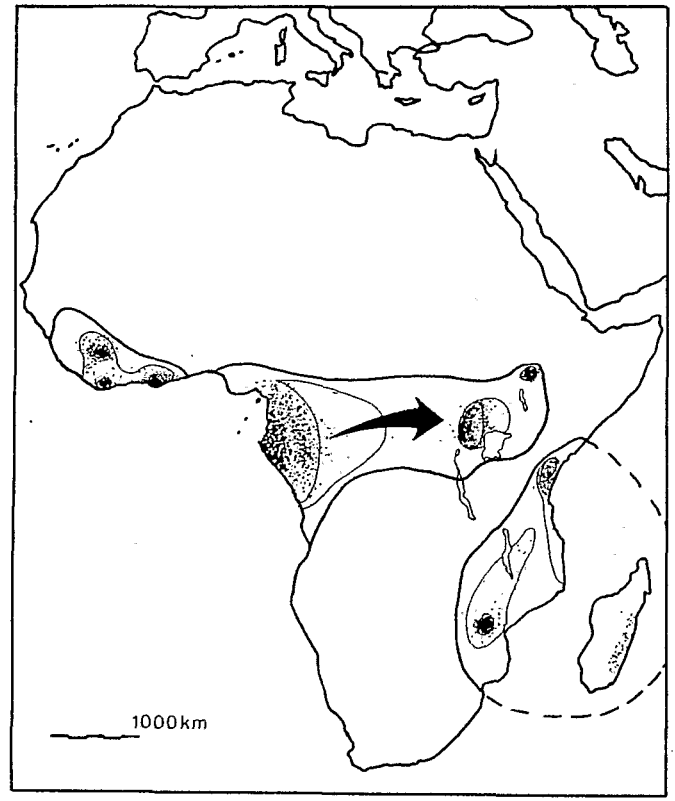


Fig. 10. Current genetic diversity and species distribution. The darker zones represent the richer areas either for genetic diversity or species number.

Relationships among species are deduced from biogeographic arguments, but they are supported by results described in previous sections. A cytogenetic differentiation exists between *Erythrocoffea* (*C. arabica*, *C. canephora*, *C. congensis*, *C. liberica*), and *Mascarocoffea* and *Mozambicoffea* (*C. racemosa*). East African coffee trees can be placed in one section, *Mozambicoffea*, because they share some characters. The ripening period is very short (a few weeks to a few months); the caffeine content is low, and some species are even caffeine-free (one example is the B form of *C. zanguebariae*, also named *C. pseudozanguebariae* Bridson). This characteristic was once thought to be specific to *Mascarocoffea*.[35] Now it can be taken as a proof of close relationships between the sections *Mascarocoffea* and *Mozambicoffea*. New plant collecting, morphological and genetic

studies would still be necessary to confirm the validity of this classification, offered tentatively here.

In the next section, strategies for collection and conservation of genetic resources will be developed. They rely on the genetic organisation of coffee as described in this section.

8. STRATEGIES FOR COLLECTION AND CONSERVATION OF GENETIC RESOURCES

In this section, we will use information and results already presented to define strategies for collection and conservation of genetic resources, the purpose being to make the widest genetic diversity available to breeders. Conservation is very necessary as deforestation in tropical Africa is dramatically endangering the survival of forest blocks, the natural repository of wild coffee trees.

8.1. Plant Collecting

Based upon results from previous surveys and collections, clues may be offered for continuation of collections according to specific goals.

8.1.1. In Maximum Genetic Diversity Areas

In a previous section, a maximum genetic diversity area has been shown in the Cameroon–Gabon–Congo region. This area is still little explored. Many species should be found as well as a wide diversity within each species. Species involved are *C. brevipes* and *C. staudtii*, but also *C. humilis* and many undescribed species. There is a high probability of discovering unreported species.

Another area of high diversity, also not well explored, is the region along the Mozambique–Zimbabwe border. Many species have been collected as herbarium specimens (*C. racemosa*, *C. salvatrix*, *C. ligustroides* + *C. mufindiensis*) but are not found in living collections.

8.1.2. In Special Areas

We are concerned with borders of distribution areas, contact zones between forest and savannah. *C. canephora* and *C. liberica* have actual populations in contact zones, and some in savannah zones. The variety Robusta has its origin at Lusambo (Zaire) in a savannah region. *C. excelsa* (or *C. liberica*) was found by Chevalier in a savannah zone in Central Africa. Cultivated Kouilou from the Ivory Coast also comes from savannah. Thus, a question

should be addressed: do these forms, collected in gallery forests within savannah regions, with xerophytic and heliophytic adaptations, have special characters giving them better chances for successful cultivation? We do not have an answer to this question, but this topic should be a good reason for collecting new wild coffee populations in these regions.

8.1.3. Within C. canephora

Collection may be directed towards the borders of a distribution area, or towards the centre of diversity located in the central Atlantic region from Nigeria to the Congo.

The beginnings of *C. canephora* cultivation were characterised by many inter-regional genetic material exchanges. Coffee plantations were a favourable place for natural interform hybridisation. An example is offered by the Ivory Coast coffee estates (see Chapter 5), but a comparable situation occurred in Madagascar. At the present time, on a large scale, genetic material in these plantations represents valuable new genetic combinations not found in the wild. Therefore, new explorations should be undertaken within *C. canephora* 'cultivated populations'. They should be based on a search for outstanding trees. Such a method has already been used in the Ivory Coast and Madagascar for establishing the first collections of this species. All commercial varieties distributed in these countries until now were derived from these collections through vegetative selection.

8.1.4. Sampling Methods

For the broadest diversity collection in a given area, which strategy should be selected? For a given number of collected trees in a delimited area, should we decide to take more samples per population, or should we sample more populations? Taking into account that natural populations are often small-size populations, in many cases a sampling scheme simply involves harvesting all trees of a population. Some genes are found on a very local basis. In the Ivory Coast there is a small *C. canephora* population where most of the trees are rust resistant (tests were carried out in field conditions), whereas the surrounding populations in a range of a few kilometres are sensitive or very sensitive to rust. With such a local gene distribution, many populations should be sampled.

Can we estimate the size of sample for a large population? A simple probability calculation shows that 230 trees should be sampled to have a 99% chance of collecting an allele present in this population with a frequency of 0·01. Only 30 trees are necessary if this allele has a frequency of 0·05 and if we accept a 95% chance of collecting it. Within a population, the sample size is influenced more by the prospector's choices of probability thresholds than by allelic frequencies. For us, a good compromise would be a sample size in the range of 30 to 60 trees.

8.2. Genetic Conservation

8.2.1. Current Methods

Improvements have been made in seed conservation. However, the length of conservation time does not go beyond one year[50,51] and is insufficient for long-term storage.

Thus, coffee trees are kept in living collections in forested plots which buffer environmental factors. These collections allow a medium-range conservation; for a typical individual, the lifespan in these conditions is about 50 years. Turnover in these collections can therefore be maintained at a low level, which limits losses and errors inevitably linked to the establishment of new plants.

8.2.2. Options for the Future

In vitro *cultures.* In this era of new biotechnological methods, the possibility of saving all the collections in 'test tubes' is enticing. To be feasible, it will be necessary to wait until *in vitro* propagation methods are adapted to genetic resource constraints, i.e. the ability to propagate any genotype and the ability to limit growing rate. Furthermore, in these very artificial conditions, long-term genetic stability is not yet proven. Problems from low-level technology have also to be taken into account: power failure and other technical breakdowns are a reality in every country.

In situ *conservation.* The interest of this type of conservation resides in the opportunity to preserve a higher number of coffee trees as compared with the current conservation method. However, constraints should be pointed out. At the present time, possible zones for forest reserves are very limited in number and size. They are threatened by continuous deforestation. Another problem arises from the fact that, at the present time under deforestation, forest blocks have acquired a patchwork-like distribution. This is in strong contrast to what was the rule for the preceding thousands of years of *Coffea* evolution, when forest blocks in West and Central Africa were continuous. Within an *in situ* conservation system, coffee populations will evolve as isolates, but we have demonstrated that migrations and gene exchanges are important phenomena for coffee evolution.

In the regions where *C. canephora* is found growing wild, many coffee estates have been established since the beginning of the cultivation of this

species. Thus, now, most wild coffee populations in forest remnants are surrounded by cultivated forms of the same species. As we have shown in a previous section, gene flow is active between these two types of population, and at the present time one can observe in the forest reserve the creation of 'half-bred' populations. This new genetic constitution may be a starting point for a new evolution. However, this evolution can hardly be controlled and would be far away from the goal aimed at with *in situ* conservation.

In situ conservation allows coffee population conservation but within an environment already different from that which coffee populations have experienced previously. Thus, this type of conservation supports new coffee population evolution modes. Moreover, this conservation is of interest only as an integrated action. The whole flora would be protected, and not only coffee populations. However, inventories of flora and vegetation groups are still needed in order to determine the most valuable locations for forest reserves with genetic conservation purposes.

9. BREEDING SCHEMES AND INTERSPECIFIC RELATIONSHIPS

9.1. General Approach

For breeding purposes both intra- and interspecific variability have to be evaluated and used. We have been less concerned in this chapter with intraspecific variability, because much breeding work has already dealt with this problem and developments will be discussed in the next chapters. However, intraspecific variation in the self-compatible species *C. arabica* has been been somewhat underestimated and underutilised, the main reason being lack of interesting genetic material. Now this gap has been filled and exploitation of intraspecific diversity in *C. arabica* can start. Information on work conducted by a French team with collected wild *C. arabica* coffee has been reviewed elsewhere.[52]

In this section, we will place more emphasis on diversity created through interspecific hybridisation. A main conclusion drawn from the preceding results is that all diploid species share a common base genome, even though some differentiation between *Coffea* sections is noticeable. Gene transfer between diploid species should be considered as a valuable and practical tool for diploid species breeding. This subject will receive more emphasis in a following section and in succeeding chapters.

Another possibility, gene exchange between diploid species and *C. arabica* (Fig. 11), has been the focus of greater interest in the last twenty

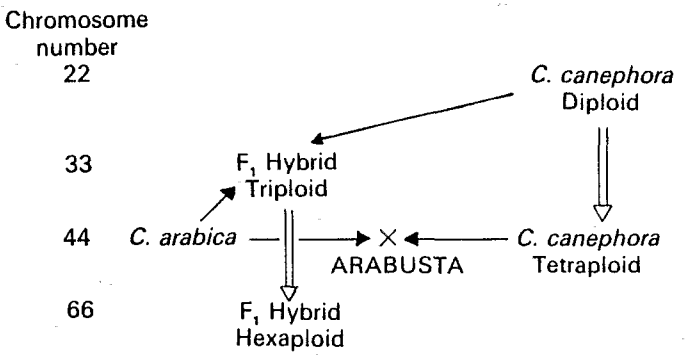


Fig. 11. Various possible routes for interspecific hybridisation between *C. arabica* and diploid *Coffea* species.

years. This method was first explored in Brazil in the 1950s.[53] A few *C. canephora* tetraploid individuals were produced through colchicine treatment, and then crossed with *C. arabica*. The hybrids were backcrossed with *C. arabica* to obtain plants with most of the *C. arabica* characteristics but retaining some of the *C. canephora* characteristics. The Icatu population has such an origin, with genetic resistance against leaf rust retained from its *C. canephora* parent.

At IRCC in the Ivory Coast the same combination was also tested. Breeding was based on the search for valuable characteristics in F_1 hybrids, those hybrids being maintained by vegetative propagation. They were named 'arabusta'[54] (see also Chapter 8). The first F_1 hybrids were very vigorous, with an interesting potential productivity, but showed some sterility problems.

In order to tackle these problems, more basic research began at ORSTOM, Ivory Coast and Madagascar, according to the following programme:

(a) Studies of diploid interspecific combinations.
(b) Study of 'arabusta-like' interspecific combinations after producing tetraploid individuals from a large gene pool of diploid species and interspecific diploid hybrids.
(c) Comparison of individuals obtained from hybrid combinations involving the same parents but with various ploidy levels. Triploid, hexaploid and arabusta hybrids were studied.

9.2. Diploid Interspecific Hybrids

Several natural interspecific hybrids have been known for a long time.[55] They were not used commercially, with the exception of Congusta. These

F_1 hybrids were produced from *C. canephora* × *C. congensis* combinations and distributed to the growers in Java. This interspecific combination was also tested in Madagascar. Through vegetative selection within progenies, several clonal varieties were distributed in that country. They had a potential productivity equivalent to that of *C. canephora* varieties and exhibited a clear resistance to waterlogging.

From the results of the interspecific studies that have been carried out for more than ten years, a better picture of interspecific combination potential can be drawn. With the exception of the Congusta hybrids already noted, F_1 interspecific hybrids are afflicted by sterility problems. Thus, their direct commercial use is seriously impaired. However, several routes towards fertility restoration are currently known. The future of these hybrids lies mostly in their utilisation as bridges between species for gene transfer either between diploid species or from diploid species to *C. arabica.*

9.3. 'Arabusta-like' Interspecific Combinations

Most of the hybrid combinations were produced without any difficulty.[56,57] Cytological studies are in progress. Fertility studies based on pollen stainability or on proportion of two-seed fruits show that slight differences can be found depending upon species participating in the combination. The average fertility of the *C. arabica* × *C. congensis* hybrid is higher than that of the *C. arabica* × *C. canephora* hybrid.[57] Two ways of breeding are possible. (i) F_1 hybrids are found to be satisfactory and can be maintained by vegetative propagation. Thus, the breeding effort will be done on progenitors with selection on interspecific combining ability. (ii) Commercial varieties need to be more arabica-like, and in this case several backcrosses towards arabica would be necessary. This second route has produced the Icatu variety in Brazil. In Kenya, a breeding programme based on these premises which has already started should produce coffee berry disease (CBD) resistant varieties.

9.4. Comparing Different Hybrid Types

Within the combination *C. arabica* × *C. canephora*, we have used many different genotypes from both species and with different ploidy levels. Doubling the chromosome number of triploid sterile hybrids produced hexaploid fertile hybrids. Based on standard parameter estimates, hexaploid hybrid fertility is higher than arabusta fertility. However, placed in a *C. canephora*-like ecological environment, hexaploid hybrids reveal a lower productivity than arabusta hybrids, which can be explained by a very low hexaploid seed filling at low altitude.

Summarising, it can be said that hexaploid hybrids with a higher proportion of *C. arabica* in their genome look and act more like *C. arabica* whereas arabustas have characteristics intermediate between *C. arabica* and *C. canephora.*[56] Therefore, hexaploid hybrids appear to be good material for transferring genes to *C. arabica.* A first backcross between these hybrids and *C. arabica* produces pentaploid plants. The next backcross produces a majority of tetraploid plants.[58]

9.5. Potential

As a whole, these results demonstrate the potential of interspecific hybridisation for coffee breeding. The accumulated knowledge shows that:

(a) It is possible to see all diploid species as belonging to the same gene pool and sharing the same base genome.
(b) It is possible to produce *C. arabica* × diploid *Coffea* interspecific hybrids. These hybrids are novel coffee forms and so deserve their own breeding programme. Moreover, these forms represent bridges between *C. arabica* and diploid species. It can be said that routes for genetic transfer from diploid species to *C. arabica* are now quite well documented.
(c) Ploidy is not a serious genetic barrier any more. Diploid species and *C. arabica* should be seen as a unique gene pool. *Coffea* genetic resources should be used to improve *C. arabica* varieties as well as *C. canephora* or other diploid species.

10. CONCLUSION

In a short conclusion, we want to point out the main facts about the genetic resources of coffee.

The most immediate result is the availability of novel genetic material for breeders. This has been made possible by recent coffee-collecting missions in Africa and the creation of living coffee collections.

Genetic resource studies were conducted as fundamental research. Based on wild collected coffees, they brought much information on the genetic characteristics of these species. Routes for gene transfer among species have been described. Interspecific combining ability has been tested. The genetic structure of coffee species is better understood and this has led to new perspectives for plant collecting and breeding schemes. Indeed new breeding schemes have their origin in these studies. This is the case for *C.*

arabica (see references 18, 59 and 60, and Chapter 4) as well as for *C. canephora* (references 30 and 61, and Chapter 5).

The usefulness of the work that has been done has been demonstrated. However, it should not be seen as complete. Advantage should be taken of information obtained from the material analysed to set up new explorations. These explorations should bring novel genetic material and perhaps coffee species still unknown. Broadening our collections requires international cooperation in the collecting phase and above all in the conservation phase to keep this material alive and to allow free access to it.

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Edited by

R. J. CLARKE

Formerly of General Foods Ltd, Banbury, UK

and

R. MACRAE

Department of Food Science, University of Reading, UK

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Preface

The present volume, Volume 4 in this planned series on coffee, takes a further marked change in technical direction from the preceding volumes on chemistry, technology and physiology to return to the starting point for coffee. This volume deals with the various aspects of the cultivation of coffee plants, which are subsumed under the general term agronomy. Two chapters (2 and 3) describe the practicalities of the cultivation and harvesting of the two main commercial species of coffee, that is, *C. arabica* L. and *C. canephora* (Robusta). These two species are available for growing, in a number of different varieties and cultivars, as a result of natural selection and of breeding programmes, which subject is again discussed according to species in two further chapters (4 and 5), but underpinned by the first chapter, which describes the genetic resources of the entire *Coffea* genus available to coffee breeders. Although they are also discussed in the foregoing chapters, breeding programmes for the highly important aspect of improved disease resistance (especially that of the coffee rust disease, mainly affecting the Arabica coffee tree) are dealt with in a comprehensive manner in a chapter entirely devoted to the subject. Certain interspecific hybrids of Arabica–Robusta, e.g. Arabusta, have been developed in recent years and have attracted attention, warranting a separate chapter, though both intra- and interspecific hybrids of various kinds feature generally in this volume. Again the subject of biotechnology is now much in vogue, so that a further chapter (7) deals with its application to coffee breeding programmes, especially through tissue culture. Finally, though not strictly agronomic, there is a related chapter on the biosynthesis of some important coffee constituents.

It is the aim of the Editors to have presented all these experimental studies and current practices, much of it not readily available—certainly not in the English language—in a way that has not been done before within the compass of a single volume devoted solely to the subject. They have been fortunate to have been able to call upon the services of many leading international experts, each specialising in a particular field.

These subjects as scientific disciplines necessarily carry a very large number of different scientific terms and words, which may need some explanation for those not familiar with them, e.g. scientists and other readers for whom these are not their primary disciplines. It is particularly noteworthy how many of these apparently obscure terms are Greek and/or Latin based, so that knowledge of their origins makes them more immediately understandable. For this reason, we have added a glossary, and also a short summary of etymological derivations of interest, as appendices.

Many of the manuscripts in this volume were directly submitted in the English language. The General Editors pay tribute to those authors for whom English is not their native tongue. Several chapters (3, 5 and 8) were translated by one of the Editors (R.J.C.), with good cooperation from the authors, for which we also thank them warmly.

R. J. CLARKE
R. MACRAE

Contents

List of Contributors

J. BERTHAUD

Centre ORSTOM, 2051 Avenue du Val de Montferrand, BP 5045, 34032 Montpellier Cedex, France

A. J. BETTENCOURT

Centro de Investigação das Ferrugens do Cafeeiro, Quinta do Marquês, Oeiras 2780, Portugal

H. R. CAMBRONY

Formerly Assistant Director of Research, IFCC, Paris, France. Present address: Le Cézanne-A, 603 Avenue du Pont Trinquat, 34000 Montpellier Cedex, France

A. CARVALHO

Secretaria de Agricultura e Abastecimento, Instituto Agronômico, Seção de Genética—IAC, Avenida Barão de Itapura 1481, Caixa Postale 28, 13100 Campinas SP, Brazil

A. CHARRIER

Centre ORSTOM, 2051 Avenue du Val de Montferrand, BP 5045, 34032 Montpellier Cedex, France