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From the Olive Flower to the Drupe: Flower Types, Pollination, Self and Inter-Compatibility and Fruit Set

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1. Introduction

1.1. Frame of the chapter

Although the tree development directs the olive tree yield we focus here to describe the main phases, stages and key steps from blossoming to harvest. The olive tree produces much more flowers than all other trees, however, fruit set and final harvest are limited by several parameters.

Most articles in literature deal with the physiological aspects of the transformation of the olive flower into the drupe. Little attention has been given to genetic involvements that underlay the physiology and the biochemistry. A comprehensive survey of the literature as much as we can is given with genetic commentaries based upon recent genetic progresses made in the field.

The olive tree derived from the oleaster and the oleaster belongs to an intertropical (Asian, African) genus and a major difference between northern species (*Fraxinus*, *Syringa*, *Ligustrum*) is the bare axillary buds at the basis of leaves. This explains why the olive is more susceptible to freeze than other Oleaceae as *Fraxinus*. Olive flower buds appear on one-year-old twig in terminal position or at the axillary of the leaves.

2. Blossoming induction

2.1. Wood bud versus flower bud

There are many disputes in literature to determine when the induction takes place and when differentiation between vegetative and reproductive bud occurs. The first cytological traces of differentiation between the two types of buds occur in February –March [1] in the north hemisphere that infers the physiological signals had triggered the mechanism early, and

physiologists agree the induction takes place about the preceding July [2]. Guayneychya [3] from the P. Villemur' team in Montpellier has delimited the stage by sequential removal of fruits in inflorescence, and he showed that it is really induction since without enough cold in fall (November-December) the buds will not turn to flower, but stay vegetative. Conversely, if the load is excessive most buds will not further turn to blossom next year, which is responsible of alternance. All buds will not give a twig or an inflorescence. Some are quiescent without apparent reason. It appears that this depend on the age of the twig and as old it is between 1 and 5 years and the older it is the mowder the chance to give a new twig. The main buds will not maintain up to 3 years, but the supernumerary buds survive and may develop. This explains why the external branches of a tree are pending and when it is not pruned old twigs will not give new buds either for a new twig or for an inflorescence. Thus, the specific shape of an old olive tree is due to this mechanism.

2.2. Genetic basis: Gene for architecture, gene for ramification

The natural development of an olive tree from a seedling or a one year cutting leads to shape the tree depending on the genotype. Moutier et al. [4] have recorded different shapes and the tree architecture has consequence on the appearance of the first flower buds. The repartition of the flower buds on a twig the second year is also depending on the genotype: *Lucques* has the lower number of flower buds whereas *Olivi re* has the highest number.

The question is therefore whether pruning trees may change the density of flower buds. Pruning has effect to regulate alternance bearing, but not to change the fate of the buds. Deep trimming on an old tree sill suppress old twigs and enables the development of supernumerary buds.

2.3. Environmental factors: Temperature, cold, latitude, diseases

Surprisingly, the main factor that enables the development of inflorescences is temperature. Cold 7.2 C are required to induce inflorescence appearance in April-May as experimented by [1]. A long cold exposure is required to obtain the optimal level of flower buds. There also the effect is genotype dependent. Latitude has not effect on the induction of flower buds. Drought stresses do not affect the proportion of flower buds, but may affect severely fruit set by increasing the proportion of staminate flowers.

3. Types of flowers

3.1. Inflorescence structure

Either short, compact and long is the classic way to describe olive inflorescences. The type is a raceme panicle that as the leaves are at each node both opposite and decussate Obviously, long inflorescence is apparently better for fruit production, but we will see that it is not so obvious. The inflorescence shape is genotype dependent. The number of flowers per inflorescence is between 10 to 30 flowers depending upon the genotype and the position of the

inflorescence and the twig that bears it. The olive tree is characterized by bearing three types of flowers: Hermaphrodite, staminate, pistillate. Pistillate flowers – some lack stamens and most of them are hermaphrodite -- and staminate flowers that have no pistil.

Pistillate flowers are completed flowers with 4 sepals and 4 petals, two stamens and two fuse ovaries.

Some flowers may have a pistil without stamens, but they are seldom and probably due to accident in the development. In contrast, staminate flowers that do not have pistil or traces of pistil, are numerous and may be prevalent. They are results of abortion of the pistil, or the pistil was there in the enlage of bud and it degenerate rapidly [5]. They concluded from their study “It is proposed that the main advantage provided by production of staminate flowers in olive is to enhance male fitness by increasing pollen output at the whole plant level, although a relict function of attracting pollinators cannot be completely discarded.».

Varieties have stamens that may abort also more or less rapidly. They may display black anthers as *Lucques*, which is fully male sterile, *Olivièrè* that displays empty brown anthers and *Tanche* that contains pollen only in one of the stamen bag. Other variety may display more or less yellow pollen (a fine yellow powder escapes from the stamen) [6].

3.2. Function

Literature displays many hypotheses to explain the reasons for two types of flowers. To summarize most hypotheses are based on the limiting nutrient (nutrient economy) to produce more pollen without the load of producing ovules that could not be fed. Wind dispersion of pollen in all species requires a high pollen production and it is logic to think that the olive tree manages high pollen production and it saves hermaphrodite flowers. It is probably not so simple due to the diversity of flower bearing in the different Oleaceae.

Since fruits can be produced only by pistillate flowers the final load of the tree will depend on those flowers. The proportion of hermaphrodite flowers is depending upon several factors that can be organized into a hierarchy as the genotype, and the environment factors such as the position of the branch, the position of the inflorescences on the twig, and the position of the twig, the temperature at the meiosis stage [5].

Hartmann [1] has reported to see olive orchards with only staminate flowers and of course they can't produce any fruits. Thus, it is clear that the environment may determine a part of the trait. However, we noticed that selection of such variety was probably done due to quality of the fruits. However, this example seems exceptional. The amplitude of variation for the trait “ratio staminate/ hermaphrodite flowers” in one tree seems is not so high when choosing a branch with enough inflorescences. As for fruits (1kg fruits are required for oil analysis) the number of flowers to be examined has to be thousands unless the determination will be uncertain. Usually, the terminal flower(s) of one inflorescence is(are) hermaphrodite and the lateral ones may more or less staminate. Furthermore the determination has to be done at different stages following white flower bud stages due to many flowers may fall quickly leaving the hermaphrodite flowers prevalent [7].

3.3. Genetic basis of the proportion between complete and staminate flowers

Only few diversity studies lead to determine the proportion of staminate and pistillate flowers. In the Villemur team, [8] and [9],[10] have determined the proportion of each type of flowers to estimate fruit set based on hermaphrodite flowers only. From their observations the percentage of staminate flowers may vary from 95% as in *Lucques* to 5% as in *Salonenque* with little year variation effect. However, the pollen released from an olive tree is not well correlated with the proportion of staminate flowers, whereas it is better correlated with the size of the inflorescence and the numbers of flowers [11]. [11; 12;13]. Ferrara et al, [14] shows the variation in pollen production and pollen viability between varieties. Damialis et al, [15] show that *O europaea* does not produce more pollen than other tree species. Zafra et al, [16] have studied enzymes that eliminate free oxygen toxic for cells (ROS and NO) in developing anther tissue showing the activity is correlated with receptivity stages.

Consequently, cultivars are ranked based on the proportion of hermaphrodite flowers and in the whole the proportion of inflorescence leading to a fruit is about 1 and therefore hermaphrodite flowers are enough to ensure enough harvest.

Flowering period, amplitude and variation year a year

In a given place the date of full blossoming varies year a year in a range of 6 weeks. Early blossoming is reported to ensure better yield than late blossoming in the people belief. Other factors given below may modulate the harvest yield. Orlandi et al, [17] have studied several factors to model spatial pollen spread in orchards that may help olive growers to locate pollinisers in orchards.

3.4. Expectation from climate changes

As for grape the average date of blossoming is earlier than thirty years back in a given place. But the risk of climatic incident increases with the earliness of the blossoming and statistics are more or less neutral on yields, thus the effects of climate changes affect surely pathogens and pests of the olive. Late blossoming increases the risk of heat shock on flower that may prevent flowers to open, and if they open pollen may be damaged (Villemur P. Pers comm.).

3.5. Cases of related genera in Oleaceae

Wild olive or oleaster

The olive is said andromonoicous and on wild in nature trees the proportion of complete flowers to staminate flowers may vary in a wide range: 95% staminate flowers, even in some cases, complete absence of hermaphrodite flowers, whereas on some trees hermaphrodite flowers are exclusive [18]. Consequently, from this key data one can infer that selection of varieties along centuries was relatively neutral as for the proportion of staminate flowers, and thus was based upon fruits and oil characteristics

3.6. *Olea europaea* subsp. *cuspidata*

Is a related subspecies to the olive belonging to the same genus. It looks like the wild olive, and twigs of the year before bears different proportion of hermaphrodite and male flowers [19].

In *Olea* and *Fraxinus* genera both anemophilous, flowers appeared before the leaf and after the leaf, respectively, and they both carry hermaphrodite flowers without calyx as corolla and with calyx and corolla, respectively. Besides hermaphrodite flowers made of 1 pistil and 2 stamens. They both develop panicles (inflorescence) with 50 – 400 flowers from lateral one-year-old twigs. The olive tree individuals display different proportion of hermaphrodite (95 to 5%) and flowers lacking pistil (staminate flowers) (95-5%).

Other genera such as *Phillyrea*, *Jasminum*, and *Fraxinus* display different interesting and intriguing situations for the proportion of staminate and hermaphrodite flowers. *Chionanthus virginicus* is dioecious, having male (staminate) and female (pistillate) flowers on separate plants. *Phillyrea angustifolia* displays high proportion of individuals with only staminate flowers whereas the counterpart is hermaphrodite, but among hermaphrodite there are two classes called G1 and G2 that can mate each other and with the male individuals, whereas individuals belong to G1 cannot mate each other as individuals belong to G2 [20].

Fraxinus includes several species. *Fraxinus* individuals are said polygamous because they may even be different flower types within an inflorescence and the sex expression of individual trees shows a continuum of gender, i.e. they have either staminate flowers, pistillate flowers, both unisexual flower types, only hermaphroditic flowers, or a combination of uni- and bisexual flowers on different branches. The male flowers for both the olive and *Fraxinus* are always on lateral twigs. The difference between *Olea* and *Fraxinus* is that all the olive trees carry always at least some hermaphrodite flowers.

The present knowledge on flower development leads to believe that staminate flowers are due to pistil abortion determined by genetic regulation plans about one month before blossom [1]. For the olive several authors sustain that nutritional resources are insufficient to make all flowers complete, and that pollen production is prioritized because there is enough hermaphrodite flowers to ensure fruit production [5].

4. The reproduction system of the olive tree

4.1. Pollination

4.1.1. Pollen structure and organization

As for other Oleaceae the pollen grain is slightly ovoid about 20-30 μm in diameter [21]. It is not possible to identify the species which produces pollen grains in archaeological remains. Thus, Archaeologists have frequently noticed 'Oleaceae'. In most cases it is olive pollen. An adult olive tree produces about 2×10^9 pollen grains that give yellow colour to every surface around an olive grove.

Pollen transport is made by winds onto long distances – over 1000 km – but loss in pollen viability is not well analyzed and there are many divergent assertions on time and distance for loss viability. Probably, as estimated by [22] loss in pollen viability occurs after about one hour due to exposition to rays in the atmosphere [23].

Pollen wind role is very important to not only disperse and diffuse pollen grains, but also to transport them fast to reach stigma before they lose their viability. Thus, the pollen physiology is not well understood in the olive tree, and most environmental factors – hot temperature stress, rain, remain to be studied in details to explain variation in fruit set when pollination has appeared deficient [24].

4.1.2. Pollen sterility types are numerous in the olive cultivars

The main type of male sterility is determined by the mitochondria of the CCK cytoplasm [25]. The anthers are empty and if some pollen seems present it is fully sterile, but it may exist some restorer alleles (Rf) encountered in the wild olive trees in North of Africa [26]. In some environmental conditions the olive trees carrying this cytoplasm, which gives the cytoplasmic male sterility (CMS) may produce a few of yellow powder, but the pollen is never functional. *Olivière* a variety from France (Languedoc-Roussillon) displays such CMS. Varieties carrying this CMS are vigorous as Chemlal de Kabylie due to probably to the economy they make in pollen production.

Lucques, a table variety from France (Languedoc-Roussillon) displays empty anthers and is characterized by dark anthers. *Lucques* display a high proportion of staminate flowers and thus the pollination has to be very effective on the few hermaphrodite flowers to ensure fruit sets.

Tanche a table variety from France (Rhône - Alpes Region) displays a partial sterility (about one bag of the anther is full of pollen per flower) that infers olive growers have to help pollination by adding polliniser varieties that are included in the appellation “Olives de Nyons”.

4.2. Pollen and archaeology

The Oleaceae pollen in Egyptian tombs, in sediment of lakes have been widely used to trace the colonisation of the Mediterranean basin by the oleaster [27] and to trace the diffusion of the olive trees by Phoenicians and Romans. However, due to the long displacement by the wind some reserves are made on these studies.

4.3. Pollen and allergy

The most common effect of the olive pollen is its allergenic effect on about 30% of the population, but most people ignore that is due to the olive pollen. About ten allergens have been characterized [28] with a huge diversity in the responsible proteins [29,30]. Many researchers forecast that plantation of the olive trees for decorative purposes in the towns is not neutral to the populations and should be probably limited to reduce the allergenic effects in the future. Many researches dealt with the allergens to understand from an evolution point of view the

reason why the Oleaceae have developed so many allergens in the pollen grains (Ash tree, Lilac, Privet, Jasmines, Phillyrea, and so on). Is there interference between allergens and S-alleles in varieties? Although the role of allergens in the pollen is not well understood, their variation described at protein level seems huge and not corresponding to clear cut classes as S-alleles. However, because the physiological mechanism involved in pollen-stigma reaction is ignored yet, it is too early to respond to this question.

4.4. Pollen to forecast harvests

Pollen grains from many plants and in peculiar from the olive tree are trapped in filters from the atmosphere to forecast the epidemic peak in the populations [31]. Moreover, researchers have developed models to forecast olive harvest based on pollen grain density in the atmosphere [14]. The method is running in different countries (Italy, Portugal, Spain and Tunisia) [32].

Indeed, the pollen should land in most of stigma from the olive tree and because there is no attractive effect but only chance that a pollen grain lands on an olive stigma, one may think that is the reason to explain the abundant pollen production by the olive tree. For other fruit trees (Peach, Apple, Cherry) bees and wind can transport the pollen and due to bees visit only flowers from the same species during its journey, the exchanges of pollen are targeted onto the same species. It is not the case for the olive.

5. Style organization

5.1. Stigma structure and organization

The size of the stigma (a cone, see pictures from [33 34] even small for us, is enough feathered once opened to capture about 20,000 pollen grains [14, 35]. The Italian team has made deep studies in pollination between trees in an orchard and their data are very important to understand what may occur at these stages.

Stigma receptivity is probably a stage difficult to surround because fruit set in the olive tree is very peculiar. In any case, most hermaphrodite flowers will not give fruits whatever the proportion of hermaphrodite flowers. Recently, gene expression studies have throw some light on this key stage showing the role of oxygen bound enzymes that reveal whether the stigma is receptive or not. From literature data, by hand pollination studies the receptivity of the stigma appears to be a week, but some varieties as *Lucques* displays a shorter while by about 3-4 days, that may cause poor fruit set if pollination does not coincide with pollen receptivity.

Pollen stigma interaction revealed strong metabolism intensity. Several Key enzymes have been studied during different stages of pollen stigma interaction [36]. Peroxidase, esterase, and acid phosphatase activities are considered as signs of stigma receptivity. However, data on RNase activities in the pollen tube growing, although its absence is considered as evidence for pollen compatibility in the frame of the GSI model (see paragraph below), are not con-

vincing since in other species harbouring a GSI type as in Solanaceae, the RNase activity is omnipresent, even in inter-compatible pollen.

Many questions remain without response on the quality of the pollen that has landed on the stigma. The quality means two things: 1) whether the pollen is able to germinate by itself if all other conditions enable it to do so. 2) whether the pollen and the style reaction is compatible because of the self-incompatibility mechanism that exists in the olive. If the pollen - style reaction is not compatible – it is said cross-incompatible- then the pollen tube will be blocked and further destroyed before it reached the embryonic bag.

6. Pollen style interaction

6.1. Physiology in the style

The first role played by the olive style is to hydrate pollen grains to enable them to germinate. Thus, there is probably no regulation by the stigma for pollen germination at this step. However, little is known on which pollen grain may germinate and several observations suggest that several can germinate, but only one reaches the embryo bag region [8, 10, 34]. Temperature has been shown to influence interaction [37]. The olive carries a strong self-incompatibility system, which also exists under different forms in other Oleaceae, and the hermaphrodite and male flower proportion is widely variable both in the crop and the wild [8, 18].

The self-incompatibility locus in other plants directs several genes that enable pollen stigma recognition and then pollen tube growth until the ovule. We would just stress the main features of these systems to lead readers to question about the mechanism in the olive.

6.2. Genetic bases for self-incompatibility and inter-incompatibility

Either one pollen grain harbours on the exine and the pollen tube one of the two determinant of the S-locus (each S-allele specifies one determinant: a trans-membrane protein with more or less glycosyl- radicals) it is called the gametophytic type (abbreviated GSI) or the two determinants; it is called the sporophytic type (abbreviated SSI).

The GSI type is present in Solanaceae, Plantaginaceae, Rosaceae and Papilionaceae where the incompatible pollen tube undergoes a programmed cell death (PCD) or apoptosis and disappeared in few hours [38, 39, 40]. Researchers working in the olive have a priori (probably due to other fruit trees belonging to Rosaceae harbour such GSI type) considered the olive tree belongs to this type, but in our opinion there is no convincing evidence to sustain this model for the olive tree [41].

The GSI type also exists in some other plant families without RNase activity as in Papaver [42], and the IIC reaction occurs on the style in one second due to a strong flux of calcium. In Gramineae [43], Papilionaceae and Chenopodiaceae with the GSI types the mechanisms does not involve an S-RNase, and little is known in these species. Many S-alleles have been sequenced in *Prunus* species (Rosaceae) [44] and if the size of the S-allele varies between 100

to 350 Kb, for the allele expressed in the pollen as to the one expressed in the style side, little is understood on the exact reaction leading to ICO and IIC between the pollen and the style.

The GSI model has genetic involvements that have to be exposed:

1. a *SvSw* plant cannot self-pollinate
2. it cannot be pollinated by pollen grains carrying either *Sv* or *Sw*.
3. the mutation of *Sv* or *Sw* to *S0* may lead to a self-compatible allele. *S0* is the conventional name given to a self-compatible allele.

The SSI type exists in Brassicaceae and Asteraceae. The pollen grain harbours the two determinants, thus leaving dominance relationships between the two *S*-alleles. We would stress that even if the pollen grain genome carries only one of the two alleles (*Sx* or *Sy*) on the surface of the exine and the pollen tube both *S*-allele products (*Px* and *Py*) are present on the pollen coat leading to the self-incompatibility reaction if pollen and style carry the same allele. In Brassicaceae, the self-incompatible pollen grain triggers a cascade of events leading to PCD in a few hours. This cascade involves a protein kinase with a SRK-motive (SRK-protein kinase) that adds a phosphorus to a template protein whereas if self-compatible there is no reaction [45].

The dominance relationship between two *S*-alleles makes (as example *Sx*>*Sy* means *Sx* dominant over *Sy*) that the recessive determinant of the pollen is hinted and consequently the same determinant on the female side on the stigma or in the style cannot pair with it, that leads to its acceptance as compatible. This feature makes the genetic involvements of the SSI model much more complex than with the GSI model [46].

1. A *SxSy* plant cannot self when *Sx* and *Sy* are both equivalent.
2. However, when *Sx* is dominant over *Sy*, *SxSy* can self-pollinate.
3. The mate between ♀ *SySz* and *SxSy*♂ will be inter-incompatible (IIC) with *Sy*=*Sx* and inter-compatible (ICO) with *Sx*>*Sy*. Consequently, dominance relationships favour cross-compatibility, but in the other direction ♀ *SxSy* and *SySz*♂ with *Sy*=*Sz* the cross will be IIC whatever the dominance relationships between *Sx* and *Sy*.
4. As the intensity of dominance may vary between pair wise combination of *S*-alleles from 0 (no dominance) to 1 (full dominant), consequently the intensity of self-pollination varies from 0 (no self-pollination) to 1 (self-pollination equivalent to free pollination).
5. The self-incompatibility behaves as a quantitative trait and consequently, one must define a threshold to rank varieties between self-incompatible (S-I) and self-fertile (S-F) [47].
6. Other involvements will be considered in the fertilization section.

Several recent reviews on GSI and SSI models can be consulted by readers [49, 45].

The GSI model infers that the pollen must germinate and start pollen tube growth before undergoing PCD if it is S-I or IIC. Pollen grain germination observations sustain this view, except in the *Papaver* model where the incompatible pollen grain does not germinate. The SSI model infers the expression of the SRK-kinase is constitutive in the style and thus the

pollen tube may not grow longer and disappears fast. The recognition of the pollen tube and its growth should enable to state whether the reaction is incompatibility versus compatibility. In practice, it is not so obvious due to all pollen grains do not behave as the same way [50, 51, 52, 53].

6.3. Methods to estimate pollen stigma interaction

The microscope method to follow pollen tube growth till the ovule requires specialization of peoples to make observation, it is time consuming and thus expansive [51, 8, 10]. For seed plants such as sunflower self-sterility is measured by surrounding one head with a pollen-proof paper bag and leaving another head free pollinated. The ratio of both fruit sets enables to estimate S-I versus S-F [54]. For the olive the paper bag method runs efficiently, but the number of flowers under the bag should be consistent with significant statistics on fruit set and the paper pollen proof should be of good quality [55, 56]. In fact, the main concern for researchers is the source of the pollen, which can be in the orchard or much far and thus the pollen requests to be moved. Dry pollen appears to loose germination ability fast, whereas after freezing it may be kept over months [57].

Many studies have been undergone around the stage of the style waiting for the pollen and on the pollen waiting to land on the stigma. One original study by [58] dealt with calcium behaviour in the stigma, the style and around the embryonic bag. The calcium accumulation is probably correlated with the pistil maturity and receptivity.

6.4. Advantages and drawbacks

The advantages of the paper bag method are that several controls can be performed in one orchard, and thus, the number of parameters under variation is much estimated. However, to move pollen requests several controls to verify whether the pollen does not suffer from transportation. It is not so easy to verify and thus this point enhances the number of request controls. Villemur recommended freezing pollen, others prefer to move pollen in paper bag, or to move branches. All these methods are not neutral versus results and may explain the wide range of answers on crosses between olive varieties.

In any way the self-incompatibility is quantitative and thus requests to define thresholds to rank varieties. Probably, literature records on self-fertility and self-incompatibility on the one hand and inter-compatibility and inter-incompatibility between given pairs of varieties diverged due to position of thresholds that are not given in records. To be accurate it is required to add the threshold.

6.5. Unravelling S-alleles composition in the olive

Breton and Bervillé [46] have attempted to fill the gap for some varieties between published tables on successes and failures on self-fertility and self-incompatibility and for mates of pairwise combination of varieties containing + and – or 0, by translating the signs by S-alleles. Obviously, the attribution is relative as long as the alleles have not been sequenced. Moreover, before reaching a satisfying model we followed plenty of wrong tracks. As an example, we

firstly supposed that self-fertile varieties (*Bouteillan*, *Cornicabra*, and *Verdale de l'Hérault*) contains one null-allele (S0) as in *Prunus* to lead to self-fertility.

Moutier's and co-workers have made the work to find better pollinisers [55, 56] – the word pollinators is devoted to animals (insects, mammals, and birds) that pollinate plants – to *Lucques* and *Olivière*. These varieties do not produce any pollen grain and should be pollinated by other varieties to ensure fruit set [25]. Consequently, the mates were not equilibrated between varieties, but due to the number of varieties involved (13), we considered this set of varieties pertinent to start the translation of + and – in S-alleles. Moreover, the series of varieties that did not mate *Lucques* was informative to state that the varieties should carry one allele at least in common to *Lucques*.

Male Female	MS	Amygdalolia	Cayon	Amellau	Tanche	Cornicabra	Salonenque	Bouteillan	VerdaleH	Arbequina	Aglandau	CMS	Picholine	Grossane	Manzanilla	Cailletier
Lucques		-	+	-		-	-	-	-	-			-	-	-	+
Amygdalolia		SI		+		-										⊕
Cayon		+	SI			+		+		-			-	-	+	
Amellau			+	SI				+								
Tanche					SI			⊖					⊖			
Cornicabra		-				SF										
Salonenque							SI			⊕	+		-	-	+	
Bouteillan			+		⊕			SF	⊕	+	-		+	+	-	
VerdaleH								⊖	SF				+	+		
Arbequina			-				⊖		+	SI			-	-	⊕	
Aglandau			+					-		+	SI		+	⊖	-	⊖
Olivière		-	+									nd	+			
Picholine			-		⊕			-	+	+	-	+	SI	-	⊕	
Grossane			-					-	+	+	-			SI	-	
Manzanilla			+				+	-		⊖	-		⊖	-	SI	
Cailletier		⊖									⊕					SF

For displaying symmetry in the table, *Lucques* and *Olivière* being male sterile their column as male is left empty.

Table 1. Rewritten from N. Moutier et al. [55,83] and Musho[8] with symmetric lines (female) and columns (male). All +- were converted into +. Cross in one direction only+, -; ⊕, ⊖: Crosses in two directions but dissymmetric fruit sets ; Symmetric fruit sets - -; or + + MS= male sterile; CMS cytoplasmic male sterile. Cases with dissymmetric fruit sets were pink circled. N.d. means not determined. SI and SF means self-incompatible and self-fertile, respectively.

Thus, the code we choose resulted from Gerstel' experiments [50] on Guayule (*Parthenium argentatum*, Asteraceae). Mates between two varieties involved 4 S-alleles coded R1 to R4. However, there are 6 possible combinations of these 4 alleles leading to draw an hexagon with the 6 pairs at the 6 poles. The first step was to define the dominance relationships between the four alleles satisfying the + and 0 cases from the data table [8, 9, 55, 56]. To do this step we based on pair wise combination of mates which leads to opposite fruit sets in both directions of crosses. The other important information comes from the variety *Lucques*, which has little efficient pollinisers and therefore was hypothesized carrying 2 S-alleles almost equivalent (R2=R3), but dominant over R1 and R4.

We constructed table 1 from Moutier's data to separate all mates that gave similar fruit sets (either +,+ or -,- in both directions) and those that gave opposite fruit sets (either +, - or -, + in both directions). Surprisingly, the same varieties were involved in differential fruit sets, this infers their genetic composition for the S-alleles had specific features that remain to be solved.

Based on the Gerstel's model the differences of fruit sets in reciprocal mates were due to the recessive alleles (here R1 or R4) which are present in the pair [50]. *Cayon* was the only variety that mates *Lucques*: we attributed the pair R1R4 with a priori R1=R4. Furthermore, we considered the pair *Manzanilla* - *Picholine*, we attributed the R1-allele to both because they gave reciprocal differences with *Cayon* that carries R1 (it explains reciprocal differences) and R2 to *Manzanilla* infers R3 in *Picholine* because they cannot mate *Lucques* (R2R3). Once done this first translation, all the others result from this one for the four alleles R1 to R4. Obviously, we were surprised to find *Tanche* with the same alleles as *Lucques* and that the four S-alleles explained the behaviour of eleven of the thirteen varieties.

Varieties studied ¹²	Other studies	S-S/S-F	S-locus	Best Polliniser
<i>Manzanilla</i> , <i>Amygdalolia</i>	<i>Manzanillo</i> ⁵¹⁻³⁵ , <i>Manzanilla de Sevilla</i> ¹⁰ , <i>Picual</i> ³⁵ , <i>Pendolino</i> ³⁵ , <i>Amygdalolia</i> ⁵³	S-S	R1R2	R4R4-R5R5
<i>Picholine</i> , <i>Arbequina</i> (<i>Barnea</i> , <i>Corregiola</i>) ¹⁰		S-S	R1R3	R4R4, R5R5
<i>Cayon</i>	<i>Corniale</i> ⁸ , <i>Koroneiki</i> ^{9,10}	S-F	R1R4	R5R5
<i>Grossane</i>		S-S	R1R5	R4R4
<i>Lucques</i> , <i>Tanche</i>		S-S	R2R3	R1R1,R4R4, R5R5
<i>Bouteillan</i> , <i>VerdaleH</i> , <i>Kalamata</i> ^{9,35} , (<i>Mission</i> , <i>Kalamata</i> , a- <i>Pendolino</i> , a- <i>Olivière</i> , <i>Cornicabra</i>	<i>Kalamata</i> , <i>Verdale</i> , <i>UC13A6</i> , <i>King-Kalamata</i> , <i>Katsourella</i>) ⁷⁸	S-F	R2R4	R1R1-R5R5
<i>Aglandau</i>		S-S	R2R5	R1R1
<i>Belgentier</i> ¹³	<i>Sevillano</i> ²⁴	S-F	R2R6	R1R1, R4R4
<i>Amellau</i>	<i>Ascolano-Tenera</i> , <i>Frantoio</i> ³⁵	S-F	R3R4	R1R1
<i>Salonenque</i>		S-F	R3R5	R1R1-R4R4
<i>Cailletier</i>	<i>Cailletier</i> ⁸	S-F	R4 R5	R1R1

Table 2. Summary of S-alleles attributed to 39 olive varieties

To go further with the other varieties *Aglandau*, *Grossane*, and *Salonenque*, we attributed a new S-allele R5 and we computed all dominance relationships with the other alleles to fit experimental data. Rapidly, we realized that the model was predictive. To attribute S-alleles to *Aglandau*, because the mate *Lucques* x *Aglandau* is lacking we considered the cross *Tanche* x *Aglandau* that failed and revealed that *Aglandau* carries R2, consequently *Aglandau* is R2R5. *Picholine* and *Grossane* gave similar patterns with all varieties except with *Aglandau* that sustains they carry R1R3 and R1R5, respectively. *Salonenque* and *Grossane* have also similar

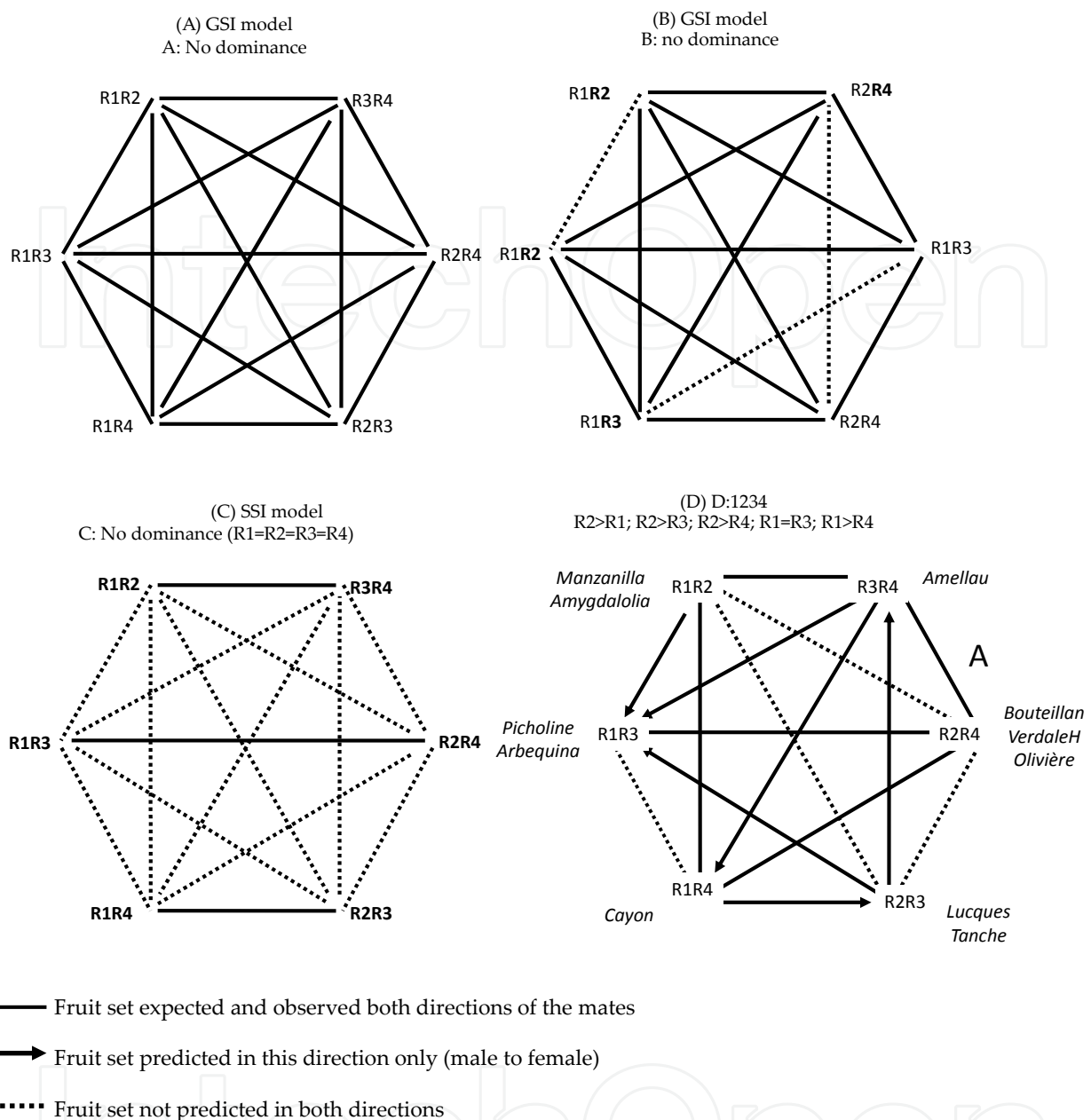


Figure 1. A: Prediction of fruit set for the six pair wise combinations of the R1, R2, R3 and R4 S-alleles in the frame of the GSI model. B: examples of mates that succeed or failed in the frame of the GSI model. C: Prediction of fruit set for the six pair wise combinations of the R1, R2, R3 and R4 S-alleles in the frame of the SSI model without dominance relationships between the S-alleles. D: application to data from Moutier et al.12

patterns except with *Cayon* and *Manzanilla*, which have R1 in common, thus *Salonenque* carries R3R5 and *Grossane* R1R5. Even if *Cailletier* has not enough mates, because it mates *Lucques* it cannot carry R2 or R3. However, *Cailletier* gives reciprocal differences in fruit set with *Aglandau* (R2R5), consequently *Cailletier* should carry a recessive allele in front of R5. *Salonenque* R3R5 cannot self-pollinate as intensively as *Cailletier*, thus we decided to attribute R4R5 to *Cailletier*. It mates *Amygdalolia* (R1R2) [8] that is in agreement with R4 and R5. *Cailletier* is *Taggiasca*

in Italy. We should apply the parsimony principle to not proliferate S-alleles. Musho [8] has also shown that *Belgentier* can mate *Manzanilla* as *Sevillano* [51] although *Belgentier* cannot mate *Lucques*. All together these data show that *Belgentier* carries R6 with $R6 > R2$, and this allele is also present in *Sevillano*.

The model was constructed on crude data provided by different authors without taking account that in controlled crosses fruit sets result from both self-pollination and cross-pollination. Depending on the pair of S-alleles the self-pollination rate varies. Now, it is possible to experiment more accurately taking into account the self-pollination rates determined under paper bags and to deduce this rate from fruit sets (Table 2, Figure 1).

7. Interests of the results

Breton & Bervillé [46] have listed some other cultivars (see Table 5 in [46]). Wu [59] provided a complete diallel design for 5 varieties, but they carry only 3 pair wise combinations of S-alleles, however, they enabled to us to attribute $R1R2$ to *Pendolino* and *Picual* $R3R4$. Many other publications provide mate results between olive varieties. However, authors did not provide enough information on mates (threshold, fruit sets,) making difficult the translation of the data in S-alleles.

Probably the list of varieties with deciphered S-alleles will grow rapidly for the benefit of researchers and olive growers, firstly, to enable diallel design between compatible varieties to check the importance of other factors (coincidence in blossoming, stigma receptivity, ovule receptivity longevity, other compatibility troubles,...) and to check at the style level whether all compatible pollen grains may germinate or if only some may germinate. These questions raise from eventual competition between pollen grains. Furthermore, the model enables to choose pollinisers a priori for a set of varieties.

Among about 20,000 pollen grains that land on a style many questions are still without answers. How many came from an inter-compatible tree [33, 60, 61, 62]. Now it is possible to design experiments to answer to such questions and to check whether competition between pollen grains does exist, not only between inter-incompatible and inter-compatible, but merely between inter-compatible pollen grains. Many observations sustained that only one pollen tube is detected in the style nearby the embryonic bag and only one reaches the ovule. This mechanism is not due to inter-incompatibility, but to some competition mechanism, which may screen among pollen grains based on still unknown parameters.

8. Fertilization

8.1. Strange features

The first feature at this stage is that even the distance between the style and the ovule is short, probably by less 2 mm, it is surprising that the pollen tube may reach the embryonic bag

between 2 to 6 days after pollination [8, 9, 51, 36, 41]. In other species the pollen tube growth is fast as in *Petunia* fertilization occurs in the next two days and the style is very long (2-3 cm) in comparison to the olive. Probably, there are some regulation steps to unravel at this level.

The second main feature is the poor efficiency in fruit set in comparison to the number of hermaphrodite flowers [63, 64, 65]. Which is the mechanism which screens among the new fruits to cause the fall of some? Only nutritional resources allowance by the position in the twig, the branch, the side of the tree, and so on seem difficult to admit. We may suggest that the delay in fertilization may cause later fruit fall, and that the early fertilized ovules are more solid than those fertilized later.

8.2. Fruit fall

Fruit fall is erratic from early fertilization and for 6-8 weeks, thus it is important to notice fruit sets under paper bags after this period unless the risk is to overestimate fruit sets. Fruit falls seems programmed in the olive and thus we suggest a working hypothesis: is there linkage between the S-locus and lethal genes leading to seed abortion?

In most species with high heterozygosity levels revealed by many studies with molecular markers to study the neutral genetic diversity, self-pollination decreases drastically not only the vigour of the plants but also the fitness that is the number of descents per female (Maize, Sunflower, other out-crossing species). The self-incompatibility model in the olive admits self-fertility in most cultivars that may enhance homozygosity in progenies. Consequently, since we have not revealed yet homozygosity at the S-allele locus, we suggest this mechanism to eliminate these individuals.

9. Fruit development

We just address here which consequence may have the fertilization process on fruit development, but not all the aspects in quality of the compounds that did not result from the compatibility mechanism.

9.1. Different phases

The increase in size of the drupe is due to two different mechanisms. The young drupe accumulates water and cells are swollen, but also cells proliferate rapidly. The first sign in fruit differentiation is the hardening of the stone because sclerenchymous cells appeared. This step is very important about 50 days after fertilization because most nutritional resources are mobilized by this stage and all other parameters slowdown at this stage. Pulp development occurs then and oil starts to accumulate [66]. Oil content as oil composition is determined by the variety with little influence of the environment whereas other compounds (Sterols, phenols,) are much dependent of the environment [67].

9.2. Parthenocarpic fruits (Shotberry)

Self-pollination and cross-pollination modify the level of seedless fruits bore by most varieties [68]. The yield in shotberries is due to self-incompatibility and or inter-incompatibility and thus it is a sign that pollination is not optimal in the orchard. Some varieties display commonly different fruit size that result from fertilized and shotberries.

9.3. Stone and embryo number

The stone contains one embryo (seed), but some may contain no seed and some contains two seeds. Studies have shown that the average weight of fruits with two seeds is higher than those with one seed and those are higher than those with no seed. The seed number is variable between varieties and may indicate more or less that not enough inter-compatible pollen has landed on the stigma [69, 70]. By choosing now a priori inter-compatible varieties, it becomes possible to determine whether this suggestion is true or not.

The embryo number is an important parameter to forecast whether the drupes will fall easily before harvest or not. The more attached drupes contain two embryos [70]. If so, to enhance efficient pollen grain number in a monovarietal orchard could be obtained by enhancement of the adequate polliniser number. In the frame of the SSI model, the deficit in efficient pollen is crucial and if adequate pollen is lacking the orchard will never produce optimal yield. [20] have suggested based on fruit sets that *Picual* pollination is deficient in solid orchards under study, but they experimented that enhancement of cross pollination did not increase fruit set.

We suggest to them to reconsider the situation by reckoning the trees producing efficient pollen grains in the frame of the SSI model. Because *Picual* is $R1R2$, based on [59] data it is probably a bit self-fertile (S-II 0.21), the rate could be calibrated in different environments (varied temperature, watering/not watering, solid: mixed orchards) in Andalusia. However, *Picual* cannot be pollinated by any pollen grain harbouring the $R2$ product (we suggest to call it $P2$) on its surface – that means it was produced on a variety carrying $R2$ (as *Manzanilla*) and $R1$ (as *Picholine* $R1R3$ and *Cayon* $R1R4$), but this infers $R1 > R4$, unless $R1$ is masked by a dominant allele as in *Salonenque* $R3R5$ –, but *Picual* is mated by *Arbequina* ($R1R3$) and *Cornicabra* ($R2R4$). Even with dominance as $R2 > R1$, there is debate for *Manzanilla* that is given as self-fertile in Spain, but behaves as S-S in most other countries [71, 72] see discussion in [46, 73, 74]. Supposing it is the same *Manzanilla* carrying the same allele pair, [51] Bradley & Griggs (1963) have shown the effect of high temperature on the expression of S-I in this variety. This may explain the variation observed for a tree for the different quarters exposed to north and south as examples [37].

10. Storage compound accumulation

The different genetic origins of the flesh and the embryo make deep differences in compounds accumulated in these organs. The drupe tissues are of maternal origin and thus they all have the same genetic composition. Oil accumulated in the flesh has the same composition for all

drupes of a variety (no genetic variance), but it may exist environmental effects. For phenol compounds the variation is important and is exploited through the appellations (AOP, DOP,...).

In contrast for the embryo, the genetic variability exists between fruits from two origins: firstly from the allele segregation of the mother plant, each ovule has a specific genetic composition, and second the diversity comes from the pollen grain genome, each pollen grain has also a specific genetic composition.

If the commercial olive oil should have between 55% to 85% of oleic acid, the oil in the embryo has usually less content in oleic acid (about 30%) and the major fat is the linoleic acid as in the sunflower oil. However, the fatty acid are not free but each esterifies a alcohol radical from glycerol and the different isomers are used to recognize appellations [68, 75].

At maturity the fruit composition are none influenced by the incompatibility system and are not addressed here.

11. Paternity tests used to unravel S-I mechanisms

Researchers have tried to determine the father (the tree giving the pollen) of the fruits of one variety to look for better pollinisers. The problem is present and the molecular markers, now common to identify olive varieties, could probably enable such approach. The method in the olive has been initiated by [76,77, 78, 79], and then other publications have tried to identify the father in the progenies of different varieties, in Spain [80, 81], Italy (Unpublished) and France [82] (AFIDOL, Unpublished).

The meaning of the method is to identify the father, which has given some specific markers not present in the female tree, by its molecular pattern discriminate among those from all other olive cultivars.

11.1. Molecular markers

The tools to discriminate the father among other olive varieties are microsatellite markers widely developed for olive variety differentiation. To do that, the method consists to isolate seeds from fruits harvested on the female variety, to enable embryos to germinate in a seedling. Then for each seedling the DNA is prepared and each DNA profile using a series of microsatellite markers is recorded.

11.2. Significance, physiology and genetics

The method infers that all the possible fathers around the female tree have been analyzed with the same series of molecular markers. Consequently, the method is restricted to favourable environment, with a limited number of putative fathers.

The method is based on the effective pollination and further fertilization of the female by the male, which introduce a deep bias in the results, because the position of the male tree in the

orchard can make the pollination easiest (under wind direction as example) than for other trees.

11.3. Drawbacks

Consequently, all found fathers in the progenies are surely inter-compatible with the female tree, but in anyway there is no response for all trees that had not given progenies with the female tree. The analysis of paternity data is therefore a bit frustrating for researchers, due to all negative results have no meaning.

12. Main research tracks

Architecture key genes that direct the shape of the trees are of interest to understand the best way to prune the trees. Their identification requires a specific genetic design, namely, to set up a progeny of about 200 trees between two varieties that display different shape without pruning. For the olive tree this step requires several tens of years.

Unravelling pollen-stigma interaction remains the main objective to handle accurately the R-alleles. Several approaches should be performed simultaneously, to enhance the chances to succeed in discovering this genes. Probably, international programs will sustain such tracks for the benefits of all.

13. Conclusions

13.1. Self-sterility and allergens

Crossing data from olive allergens (ten families) and from R-allele types (ten RxRy combinations), we did not find correlation that infers probably the genes that directed both mechanisms are not linked in the olive genome.

13.2. Male sterility and self-sterility

Many olive varieties do not produce pollen (*Farga*, Spain; *Chemlal de Kabylie*, Algeria, *Lucques*, *Olivière*,...), and some are deficient in pollen production (*Tanche*, France). These varieties cannot be used as male and their uses in genetic analyses to determine which S-alleles they carry may leave some doubt on the results, unless the varieties are used in a large number of crosses as *Lucques* and *Olivière* by [55,56, 83].

13.3. Looking for new pollinisers

Mates are systematically done with the same variety as female that may decrease the efficiency in identifying the S-alleles it carries. Consequently, we recommend to design crosses in both directions as much as it can be done.

13.4. Self-sterility an inter-incompatibility

Although the genetic of the system is simple (one locus with a few alleles), the mode of expression of the SI trait is very complex leading to enable mates even when parents carry the same alleles. Future research in this direction is probably to isolate the genes and proteins involved in the mechanism [84]. However, experiments to breed the olive are required [85].

We highlight in this chapter that most consequences still unrelated between most olive fruit development troubles come from the complex reproduction system of the olive tree. More details for each point addressed could be found in [86].

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14. Summary

The olive tree displays specific features from the production of different flowers either hermaphrodite or staminate to maturation of fruits. Both types produce pollen, but more or less efficient as for female recipient. All steps are examined from a physiological and a genetic point of view to enable readers to well comprehend the key steps in flower and fruit production. The genetic system underlying the compatibility between pollen and stigma is explained in the frame of a new model, as well as the consequences for looking to polliniser trees to most varieties. Consequences in fruit set, seed development as seed fall are examined. The olive fruit is made of two parts one of maternal origin, the flesh, whereas the embryo has different genetic origins that explain some features in oil composition variation in different environments. Several hypotheses are suggested to explain the different specific features of the olive tree.

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