Genetics/Génétique

New hypothesis elucidates self-incompatibility in the olive tree regarding *S*-alleles dominance relationships as in the sporophytic model

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ABSTRACT

Most olive varieties are not strictly self-incompatible, nevertheless, they request foreign pollen to enhance fruit yield, and consequently orchards should contain pollinisers to ensure fruit set of the main variety. The best way to choose pollinisers is to experiment numerous crosses in a diallel design. Here, the genetic mode of inheritance of SI in the olive is deciphered and it does not correspond to the GSI type, but to the SSI type. It leaves *S*-allele dominance relationship expression in the male (pollen and pollen tube), but not in the female (stigma and style). Thus, a pair-wise combination of varieties may be inter-compatible in one direction (male to female, or female to male) and inter-incompatible in the other direction. Dominance relationships also explain different levels of self-pollination observed in varieties. Little efficient pollinisers were found and predicted in varieties; nevertheless, some new efficient pair-wise allele combinations are predicted and could be created. This model enables one to forecast compatibility without waiting for several years of yield records and to choose pollinisers in silico.

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RÉSUMÉ

La plupart des variétés d'olivier ne sont pas entièrement auto-incomptibles (AI), néanmoins elles nécessitent du pollen étranger pour augmenter le rendement en fruits et donc les vergers doivent contenir des pollinisers pour assurer la production de la variété majeure. Le déterminisme génétique du caractère AI n'est pas connu et donc la meilleure façon de choisir le polliniseur est de réaliser de nombreux croisements selon un plan diallèle. Ici, nous proposons un modèle du déterminisme génétique de l'auto-incompatibilié qui correspond au type sporophytique. Ce type permet des relations de dominance entre allèles du côté mâle (pollen et tube pollinique), mais non du côté femelle (stigmate et style). Cela se traduit pour un paire de variétés par la production de fruits (inter-compatibilité) dans une direction du croisement et l'absence (inter-incompatibilité) dans l'autre direction. De plus, les relations de dominance expliquent aussi les divers niveaux d'auto-fertilité des variétés. Expérimentalement, peu de pollinisers efficaces sont trouvés dans les variétés, mais le modèle permet de prédire des combinaisons d'allèles plus efficaces qui pourraient être créées. Le modèle permet de prédire l'inter-compatibilité entre variétés sans attendre de longues années d'expérimentation et de choisir les pollinisers in silico.

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1. Introduction

Self-incompatibility (SI) in homomorphic flowers is governed by one locus (The *S*-Locus) that directs two different mechanisms (Fig. 1) in plants. The *S*-locus carries several genes of unknown function. In species of the gametophytic type, the pollen grain harbors only one determinant, either the *Sx* or *Sy* allele; this explains why it was named gametophytic S-I (GSI). The *S*-locus encodes an *S-RNAse* that is released into the style in Solanaceae, Papilionaceae and Rosaceae [1]. This enzyme penetrates from the style into the pollen tube whatever the compatibility alleles and triggers a cascade of events leading to programmed-cell death (PCD) only if the pollen is incompatible [1,2]. GSI has a variant in *Papaver* without *S-RNAse* expressed in the pollen tube [3]. In the other functional mechanism, the pollen grain harbors the two determinants *SxSy*. It functions in Brassicaceae and Asteraceae [4]. Thus, it is called sporophytic S-I (SSI), that enables dominance relationships (as an example, *Sy* > *Sx*) leading to ♀*SxSy* × ♂*SxSw* being inter-incompatible (IIC) and ♀*SxSw* × ♂*SxSy* being inter-compatible (ICO) in only this direction for a pair-wise combination of cultivars [5] (Fig. 1). For Brassicaceae, an incompatible pollen grain that lands on the style triggers a cascade of events leading to PCD that involved a SRK motif protein kinase. Several hundreds of protein kinase exists in the plant genomes, but only one is involved in the events of the SSI cascade [6]. The number of *S*-alleles in one species with GSI mechanism has been found to be 40 to 60 in several *Prunus* species [7], as in *Senecio* with an SSI mechanism [8], with, for consequence in a population, that the mating availability–the number of individuals that are fertilized/the total number of individuals–is relatively high [7,8].

Most olive (*Olea europaea* Subsp. *europaea* Var. *europaea*) varieties are more or less self-incompatible (S-I), they produce little or no fruit in pure orchards, and even for those partially self-fertile (S-F), cross-pollination with pollinisers favors higher and more regular yields. Although S-I is an important trait for production of the olive tree, the mode of inheritance of the *S*-I trait is, as yet, unknown for this species. However, looking for pollinisers–pollinator is

devoted to an insect that carries pollen–is not rational and thus in most orchards irregular yields and alternate production are the major concerns for olive growers as well as for economists and market forecasts. In the olive tree, several authors have considered it is of the gametophytic (GSI) type [9–11]. The genetic structure for most varieties is the clone [12], which means all individuals are identical for all alleles, and consequently, if the two *S*-alleles are leading to SI, the orchard will produce no, or little, fruits. To give efficient pollen grains, olive growers add polliniser trees. Historically, each variety fits one or several traditional pollinisers, but researchers have developed diallel designs in most countries to look for more efficient pollinisers than the traditional ones, which pollinate one of the varieties of the countries as *Mission* from USA [13], *Manzanillo* in Spain and Israel [14,15], *Koroneiki* from Greece [16], and *Lucques* and *Olivière* in France [17–20].

2. Determination of self-incompatibility/self-fertile and inter-incompatible/inter-compatible

Practically, pollen tube progression onto the style to the ovary is the method to determine whether a plant is S-I/S-F and whether a cross is ICO or IIC. For a species, such as the olive, this method has been used [9,21,22], but it is time-consuming and thus expensive. Moreover, the germination of a pollen grain appeared a quantitative trait and authors also considered the fruit set that is easier to determine on a large scale by surrounding some branches with pollen-proof paper bags [9]. However, fruit set under the bag may be not well correlated to pollen grain germination due to other events that may affect the set up. Finally, following pollen tube growth in the style has a limit in practice and most researchers prefer to record final fruit sets to estimate S-I, even though it can be affected by other events than S-I status.

S-I is estimated by the self-incompatibility index (S-II), which is the ratio of number of fruits for a branch carrying about 200 flowers under a bag, which enables self-pollination, to the number of fruits for 200 flowers left to free pollination without the bag [14,22]. This ratio varies
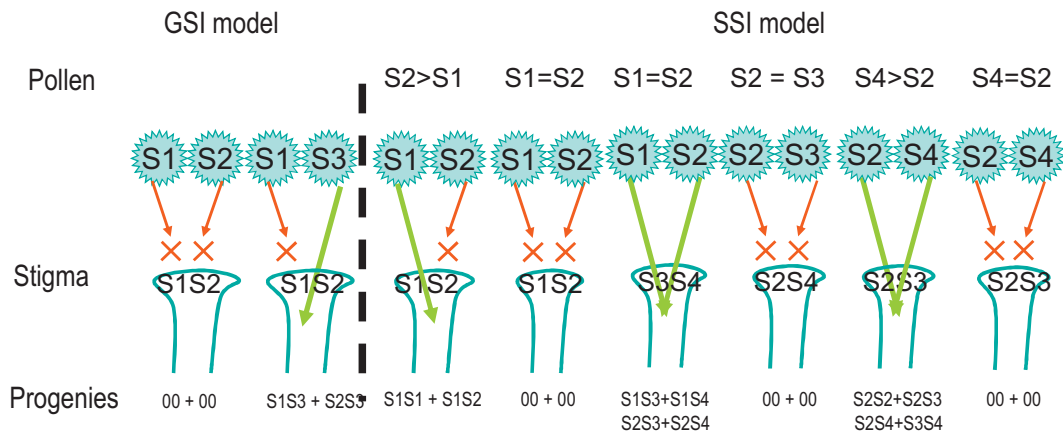

Fig. 1. Model for gametophytic S-I (GSI) and sporophytic S-I (SSI) mechanisms of self-sterility and inter-compatibility. > means dominant upon; < s recessive upon; 00 means no possible progenies. The difficult case are only represented in this figure.

from 0 (strictly S-I) to 1 (S-F), behind the threshold (10–30 fruits), a variety is S-I, and above, it is given as S-F, and for most varieties S-II is about 0.1–0.3. However, S-II was found up to 5, that indicates that most olive varieties are somewhat partially self-incompatible, and moreover, an adequate peculiar polliniser may be more efficient than free pollination. Thus, looking for such pollinisers may be attractive to enhance fruit yield [23–25].

2.1. Environment effects on self-incompatibility and flower structure

In the olive tree, some flowers have no pistil (staminate flower) and some have no stamen (pistillate flower). The proportion of perfect/complete (hermaphrodite) flowers is variety dependent. Moreover, most fruits may fall after set up and in fine only 3 to 5% of the complete flowers will produce fruits that average one fruit per inflorescence. Consequently, for most S-I studies, the fruit set in controlled cross-pollination is compared to the fruit set in open pollination. S-II appeared a quantitative trait that requires to choose a threshold to estimate S-I or S-F. Depending on the number of flowers under the bag (20 to 200) the estimation is more or less accurate, on the threshold chosen 0.1 to 0.3, a cultivar with S-II = 0.2 will change its status from S-I to S-F [17–25]. Another concern with the olive pollination comes from environmental effects on cultivar behaviors. Temperature could be the cause of this change [9]. Some are given as *S-F* in their countries (*Manzanilla*, Spain) or region (*Aglandau*, Haute-Provence, Vaucluse, and Var; *Salonenque*, Les Baux and La Crau), but are S-I in other regions as for *Manzanilla* [9–12]. However, the molecular characterization of varieties is not routine and based on morphology many synonymies may be the source of such variation [26]. Consequently, the literature displays heterogeneous results about the classi-fication of cultivars in S-F or S-I. Moreover, ambiguities may also come from methods that estimated S-I/S-F by determining pollen grain germination, fertilization of the ovaries, or fruit set, and so on. Nevertheless, whatever the methods used to examine cultivars, most cultivars are at least partially S-I and conversely, some are partially S-F. Indeed, most olive cultivars will usually set a better crop with cross-pollination especially under adverse weather conditions. Most are declared S-F varieties even although they require a pollen source from another variety [13–25].

However, olive tree pollination in orchards is the main concern for olive growers and for researchers to ensure a regular production. Experimental designs to test IIC versus ICO for varieties are varied due to many constraints in a long shelve tree species. Some authors have tried to plant trees in containers to move them easily into the neighbourhood of the female trees or vice versa, to experiment pair-wise combinations of cultivars, so as to check other factors such as the effect of temperature during pollination [9]. Most researchers have preferred to move onto the female the pollen either in paper bag once harvested or to move branches that are introduced into the paper bag that surrounded the female flowers. All these experimental techniques suffer from drawbacks due to the pollen conservation methods and to possible contaminant

pollen grains that are dispersed by wind. The pollen can be trapped onto filters and its density enables one to forecast olive yields on a large scale [27,28]–during full blossoming of olive trees in an orchard, the opening of a bag presents a risk difficult to estimate, but that can make results inconsistent on a year-to-year basis, depending on the contamination rate.

2.2. Choice of the data set

With all these constraints, to carry out a new diallel design to estimate S-I/S-F as IIC/ICO on a set of varieties appeared unrealistic. Furthermore, the literature contains enough data providing fruit sets on crosses between pair-wise combinations of varieties, frequently in both direc-tions of crosses, with all possible drawbacks listed above when the trees are not in the same orchard, that infers pollen should be displaced. However, data are relatively consistent between different authors and over years for some varieties. We attempted unraveling their differential composition in *S*-alleles to explain inter-compatibility and inter-incompatibility in the frame of the gametophytic and the sporophytic models to determine the one to which the olive may belong.

We retained IIC and ICO data from varieties of the crosses controlled by Moutier's team [17–20] and some from crosses controlled, Villemur's team [21,22,29]; both have provided consistent data over the years at the threshold of S-II at thresholds 0.3, above which the variety is S-F. Both teams have looked for pollinisers of *Lucques* and *Olivière* in the Languedoc-Roussillon region (South France) and the varieties they used have been widely described (Supplementary material, Table S1). Moutier's team has used the paper bag method, whereas Musho [17,18] has used pollen tube germination, and interestingly they used the same varieties between 1976–1977 and 2002–2009 as Moutier's team. The polliniser is declared IIC with the recipient variety when in a cross; S-II is below 0.3 for the female, and above 0.6 the polliniser is considered as very efficient. Between 0.3 and 0.6 SII may be advocated IIC or ICO depending on the case study. In self-pollination trials, the same threshold leads to S-S or to S-F. We looked for a method to convert + and 0 from Moutier's team and Musho into *S*-alleles [16–18,22–24]. However, several authors have reported, based on fruit set, strong differences–even opposite results–in reciprocal crosses for several plant species [5] and for the olive ([17] and Fig. 2). Gerstel's [5] findings on Guayule (*Parthenium argentatum* Gray, Aster-aceae) suggested that reciprocal differences should be the best way to start the conversion for two varieties carrying four different alleles (*R1* to *R4*) due to one variety having a unique polliniser among the five possible (Fig. 3, Table 1).

3. Results

For each olive tree carrying two S-alleles (*SxSy*), without dominance between the S-alleles, thus both alleles are expressed in the pollen (coded *Sx* = *Sy*) it cannot self-pollinate. This is true both in the GSI and SSI model frame. However, when *Sx* is dominant over *Sy* (*Sx* > *Sy*) then self-pollination is possible, *Sy* is hinted by *Sx*, and may lead

	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		-					◉		+	AI			-	-	⊕	
Aglandau			+				-			+	AI		+	◉	-	
Olivière	-		+			-	-	-		+		nd	+	-	-	
Picholine			-		⊕	-	+	+	-		+		SI	-	⊕	
Grossane			-			-	+	+	-		⊕		-	SI	-	
Manzanilla			+			+	-			◉	-		◉	-	SI	
Cailletier		◉														SF

Fig. 2. Rewritten from Moutier et al. [6] 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.

to homozygous *SySy* individuals that are strictly self-incompatible. This feature has two main consequences:

- a variety displays self-fertility when dominance relationships exists between the two alleles, or, in other words, one self-compatible olive variety may not carry a self-compatible allele as should be inferred with the GSI model;
- a variety should therefore display reciprocal differences when crossed with a male that carries the same recessive allele when this latter is hidden by a dominant one.

Consequently, we focused the model for the olive on an allelic series of *S*-alleles with dominance relationships. These features are detailed for several case studies Fig. 4A–F. Fig. 4A–F show that dominance relationships between four alleles make radical changes in expected fruit sets. As for *R1* = *R2*, *R3* = *R4* most crosses are IIC, Fig. 4A,B, and we introduced dominance relationships between the four alleles.

3.1. To decipher S-alleles in sets 1 and 2

Consequently, to decipher *S*-alleles we first considered *Lucques*, which is very difficult to cross with an efficient male. We supposed, based on Gerstel' hypotheses [4], that it should carry two dominant alleles named *R2* and *R3* due to the fact that it can be pollinated only by a variety carrying *R1R4* (Fig. 3A; Fig. 4E); the scheme was borrowed from Gerstel [4] to explain fruit set in the pair-wise combination of the set1 of varieties *Manzanilla*, *Picholine*, *Arbequina*, *VerdaleH*, *Tanche* and *Cayon*. Consequently, all varieties that are ICO with *Lucques* cannot carry both *R2R3*, or either *R2* or *R3* (Fig. 3A, Fig. 4A). In contrast, all pair-wise combination of varieties that are IIC with *Lucques* will share one or two of the alleles *R2R3*. *Lucques* and *Tanche* carry the same set of *S*-alleles *R2R3* (Fig. 3A). Some of these

varieties cannot cross *Tanche* and *Lucques* and they displayed reciprocal differences in fruit set, for some pair-wise combinations (Table 1), but not *Lucques* that is male sterile and the cross was unidirectional [30]. We attributed the *S*-alleles to the set1 of varieties to fit Fig. 4A. Thus, *Picholine* is *R1R3* and *Manzanilla* is *R1R2*, that infers that *Manzanilla* can cross *Picholine* due to *R2* > *R1*, whereas the cross is not possible in the reverse direction. *Arbequina* can cross *Cayon*, but not *Picholine* and *Lucques*; consequently it is *R1R3*.

The set two of varieties included *Olivière* (also male sterile) [12], *Cayon* mated *Olivière*, as *Picholine* and *Arbequina* can, whereas *Manzanilla* cannot. Consequently, *Olivière* carries the same *S*-alleles as *VerdaleH* (*R2R4*), but, as it is male sterile, we cannot verify it crossed *Arbequina*, but *VerdaleH* can cross *Arbequina* in both directions. *Bouteillan* behaves as *Olivière* as female (four crosses) except with *Grossane* and *VerdaleH*. *Bouteillan* should carry the same alleles as *VerdaleH* and *Olivière*. Consequently, *Grossane* should cross *Olivière*. This is the first misfit between the data and the model. However, more recent data obtained on a large scale verified that *Grossane* crossed *Olivière* [J. Vaisse, Pers Comm]. Thus, the misfit is eliminated.

For *Aglandau* we constructed the Fig. 4C, D with *R1*, *R2*, *R3* and *R5* (Fig. 3B, C) to verify that *Aglandau* mated *Manzanilla*, *Tanche*, *Lucques* and *Picholine*. With *R5* > *R3* *Aglandau* is ICO with *Picholine* in both directions and *Aglandau* cannot cross *Tanche*; thus, it carries *R2* and the *R5* *S*-allele. *Salonenque* cannot cross *Lucques* and *Picholine* and it carries *R5*, with *R5* > *R3*. *Grossane* behaves as *Picholine* for all varieties except with *Olivière* (*R2R4*); it thus carries *R1*, and, since as *Cailletier* it gives reciprocal differences with *Aglandau*, it carries *R5*. Thus, *Aglandau* is *R2R5*, *Grossane* is *R1R5* and *Cailletier* is *R4R5*. However, Grossane cannot cross with *Cayon*, that does not agree with the model. *Cailletier* should carry *R4* as it crosses *Lucques*, but
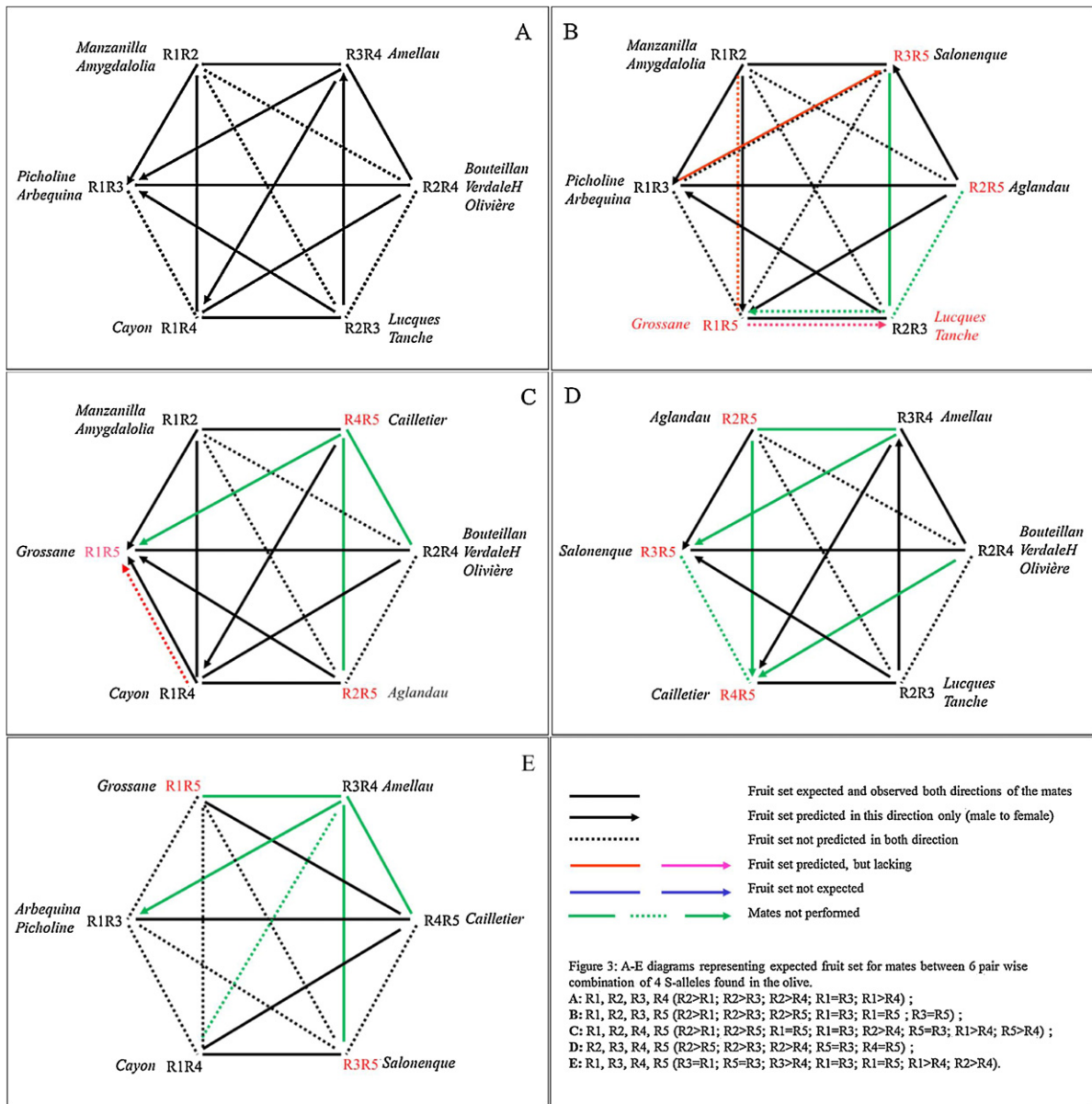
Fig. 3. **A–E** diagrams representing expected fruit set for mates between six pair wise combination of four *S*-alleles found in the olive.

considering it cannot cross with *Aglandau* (the reverse is possible) it should carry *R5*. *Aglandau* and *Cailletier* are predicted S-F. *Amellau* is *R3R4* and *Cornicabra* is *R1R2*. *Corniale* (Coil) is ICO with *Lucques* (*R2R3*) and *VerdaleH* is *R2R4*, and thus are as efficient pollinisers as *Cayon*. *Salonenque* fits *R3R5* and is ICO with *Arbequina*. *Salonenque* mates *Manzanilla* whereas *Grossane* cannot; thus this sustains the theory that *Salonenque* should carry *R3R5*. Villemur's team [22] has shown that *Manzanilla* can be crossed by *Belgentier* whereas the reverse was not possible; that infers *Belgentier* should carry another *R6* *R*-allele with R6 > R2. *R6* was also present in *Ascolano*, but cannot be deciphered in this cross [9] due to not enough mates. From crosses performed in Australia [10], *Frantoio* mates *Manzanilla* (*R1R2*), so it is *R3R4*, whereas the other mates lead to infer *Kalamata* correspond to *R2R4*, *Pendolino* and *Picual* are *R1R2*. Thus, the list of varieties with attributed *S*-alleles could increase rapidly.

3.2. Explanation of variation in the self-fertility rate

Moreover, due to the hierarchy in dominance relationships, we suggest that the more dominant allele paired with the more recessive allele will lead to the higher S-F rate: namely *R2R4*. A given pair of *R*-alleles enables one thus to predict whether the variety is S-F or S-S. *R1R2* (*Manzanilla*), *R3R4* (*Amellau*), *R2R4* (*VerdaleH, Bouteillan*), are predicted to be more or less S-F, whereas *R1R4* (*Cayon*),

Table 1

Variety pair-wise combinations leading to symmetric fruit sets (left) and dissymmetric fruit sets (right) that have enabled to decipher S-alleles attribution and enabling to construct Fig. 4. Alleles responsible of the symmetry and the dissymmetry are indicated with those involved in the reject or in dissymmetry ►◄ cross in one and reverse direction. Key pair-wise combination of crosses with reciprocal differences, compatibility (ICO) and incompatibility (IIC) in both directions. ⊕ and ◐ represent crosses in two directions but dissymmetric fruit sets.

Some key symmetric crosses		►	◄	Dissymmetric crosses		►	◄
Bouteillan	Cayon	+	+	Arbequina	Manzanilla	⊕	◐
Bouteillan	Picholine	+	+	Bouteillan	VerdaleH	⊕	◐
Picholine	VerdaleH	+	+	Picholine	Manzanilla	⊕	◐
Cayon	Manzanilla	+	+	Picholine	Tanche	⊕	◐
Picholine	Cayon	–	–	Bouteillan	Tanche	⊕	◐
Manzanilla	Tanche	–	–				
Picholine	Arbequina	–	–	Cailletier	Aglandau	⊕	◐
				Manzanilla	Belgentier	⊕	◐
Grossane	Bouteillan	+	+	Salonenque	Arbequina	⊕	◐
Manzanilla	Salonenque	+	+	Aglandau	Grossane	⊕	◐
Grossane	VerdaleH	+	+	Cornicabra	Amygdalolia	⊕	◐
Aglandau	Picholine	+	+				
Aglandau	Manzanilla	–	–				
Picholine	Grossane	–	–				
Cayon	Grossane	–	–				
Manzanilla	Grossane	–	–				
Arbequina	Grossane	–	–				
Salonenque	Grossane	–	–				
Amygdalolia	Cornicabra	–	–				

R1R3 (*Picholine*, *Arbequina*), and *Tanche* (*R2R3*) are predicted to be strictly S-S. This prediction matches the *Bouteillan* and *VerdaleH* pair. Then, we expected *R3R4* less S-F than *R1R2* pair. The most S-S pair should be *R2R3* both highly dominant. *R1R3* is expected S-S, there is no dominance relationships in the pair. Still, we propose a logical explanation for variation of self-fertility levels. In this set it fits data, but it can be widely verified with data from other teams, and if it fails it must be rejected.

3.3. The whole model

The whole possible pair-wise combination for 15 cultivars displays, 225 (15 × 15) possibilities, but because some share the same S-allele pair, we considered 5 S-alleles. However, *Manzanilla* in the model displays self-fertility that may explain that *Cayon* to *Manzanilla* was noted ICO by confusion of self-fruit set with cross-fruit set, but in the model it is IIC. Consequently, the data from Moutier should be reconsidered with the deduction of the S-F rate, when the model predicts self-fertility. The two crosses *Manzanilla* × *VerdaleH* in both directions were not performed, but *Manzanilla* IIC with *Olivière* was verified. Except with this misfit and the two lacking crosses all other pair-wise combinations of fruit set matched model prediction. Consequently, the probability that six crosses in each direction fitted the model due to chance only, is weak. We verified dominance relationships between *R1*, *R2*, *R3*, and *R4* in 28 of the 30 occurrences (Fig. 3A), but we estimated that we have enough crosses that involved *R5* (as male and as female on 30 occurrences) to verify the correct dominance relationships with all other S-alleles.

4. Discussion

When considering confidence in the model, obviously the interest of Fig. 5 is to predict cross results and to introduce logic into cross results that could appear erratic. Nevertheless, the results could serve to other varieties if researchers refer in the same testers. The varieties conserved at Inra have been characterized with molecular markers [26] and are available. Probably, there exists several clones for *Manzanilla*, the provenance from Montpellier has been introduced by G. Piquemal, P. Villemur (Pers. Comm.) and is referenced as *Manzanilla dos Hermano* [26]. For further enlargement of Table 2 with more S-alleles some crosses appeared strategic to attribute S-alleles to a cultivar as a male. With *Lucques* if VarX is ICO, this eliminates *R2* and *R3*. Thus, VarX could be *R1R4* or *R1Rx*, or *R4Ry*. VarX mates *Cayon* and *Picholine*, which enable one to attribute the correct *R1* and *R4* alleles, but *Rx* and *Ry* will remain to be identified. The literature frequently reported crosses with *Picholine* as male. Fig. 3 shows that if the cross is ICO, the variety has to be *R2R4*, *R2Rx*, *R4Ry* or *RxRy*.

Thus, the results from Moutier's team that *Grossane* was not ICO with *Cayon* appeared illogical. When a cross is found IIC, whereas theoretically it is ICO, this suggests that there is another reason that S-I prevents pollination to occur, such as the coincidence of blossoming between the two varieties–the state of the pollen brought in the bag, the late stage of the female flower–a too hot temperature in the bag, and so on. In contrast, if the cross is found ICO when expected to be IIC, this means an error has occurred. The main sources of errors should be:

- self-pollination that may be enhanced by mentor effect of the foreign pollen under the bag;
- contamination by surrounding pollen that may prevail over self-incompatible pollen.

Several features merge from controlled crosses in the olive:

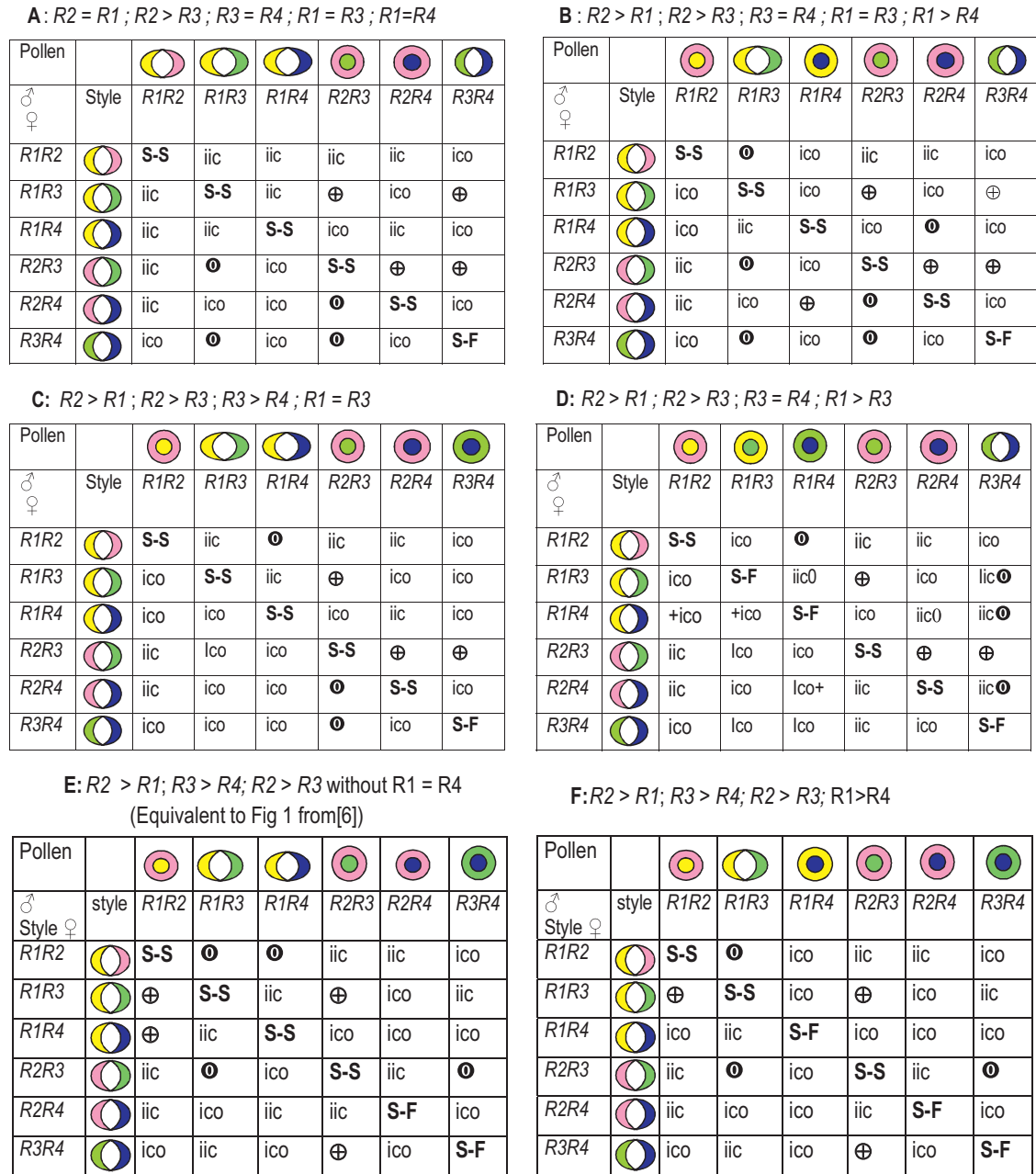- self-pollination in a bag leads to weak fruit set for most cultivars;

A: *R2 = R1 ; R2 > R3 ; R3 = R4 ; R1 = R3 ; R1=R4*

♂ / ♀	Style	R1R2	R1R3	R1R4	R2R3	R2R4	R3R4
R1R2		**S-S**	iic	iic	iic	iic	ico
R1R3		iic	**S-S**	iic	⊕	ico	⊕
R1R4		iic	iic	**S-S**	ico	iic	ico
R2R3		iic	⓪	ico	**S-S**	⊕	⊕
R2R4		iic	ico	ico	⓪	**S-S**	ico
R3R4		ico	⓪	ico	⓪	ico	**S-F**

B: *R2 > R1 ; R2 > R3 ; R3 = R4 ; R1 = R3 ; R1 > R4*

♂ / ♀	Style	R1R2	R1R3	R1R4	R2R3	R2R4	R3R4
R1R2		**S-S**	⓪	ico	iic	iic	ico
R1R3		ico	**S-S**	ico	⊕	ico	⊕
R1R4		ico	iic	**S-S**	ico	⓪	ico
R2R3		iic	⓪	ico	**S-S**	⊕	⊕
R2R4		iic	ico	⊕	⓪	**S-S**	ico
R3R4		ico	⓪	ico	⓪	ico	**S-F**

C: *R2 > R1 ; R2 > R3 ; R3 > R4 ; R1 = R3*

♂ / ♀	Style	R1R2	R1R3	R1R4	R2R3	R2R4	R3R4
R1R2		**S-S**	iic	⓪	iic	iic	ico
R1R3		ico	**S-S**	iic	⊕	ico	ico
R1R4		ico	ico	**S-S**	ico	iic	ico
R2R3		iic	Ico	ico	**S-S**	⊕	⊕
R2R4		iic	ico	ico	⓪	**S-S**	ico
R3R4		ico	ico	ico	⓪	ico	**S-F**

D: *R2 > R1 ; R2 > R3 ; R3 = R4 ; R1 > R3*

♂ / ♀	Style	R1R2	R1R3	R1R4	R2R3	R2R4	R3R4
R1R2		**S-S**	ico	⓪	iic	iic	ico
R1R3		ico	**S-F**	iic0	⊕	ico	lic⓪
R1R4		+ico	+ico	**S-F**	ico	iic()	iic⓪
R2R3		iic	Ico	ico	**S-S**	⊕	⊕
R2R4		iic	ico	Ico+	iic	**S-S**	iic⓪
R3R4		ico	Ico	Ico	iic	ico	**S-F**

E: *R2 > R1; R3 > R4; R2 > R3 without R1 = R4*
(Equivalent to Fig 1 from [6])

♂ Style / ♀	style	R1R2	R1R3	R1R4	R2R3	R2R4	R3R4
R1R2		**S-S**	⓪	⓪	iic	iic	ico
R1R3		⊕	**S-S**	iic	⊕	ico	iic
R1R4		⊕	iic	**S-S**	ico	ico	ico
R2R3		iic	⓪	ico	**S-S**	iic	⓪
R2R4		iic	ico	iic	iic	**S-F**	ico
R3R4		ico	iic	ico	⊕	ico	**S-F**

F: *R2 > R1; R3 > R4; R2 > R3; R1>R4*

♂ Style / ♀	style	R1R2	R1R3	R1R4	R2R3	R2R4	R3R4
R1R2		**S-S**	⓪	ico	iic	iic	ico
R1R3		⊕	**S-S**	ico	⊕	ico	iic
R1R4		ico	iic	**S-F**	ico	ico	ico
R2R3		iic	⓪	ico	**S-S**	iic	⓪
R2R4		iic	ico	ico	iic	**S-F**	ico
R3R4		ico	iic	ico	⊕	ico	**S-F**

Fig. 4. Key to explain reciprocal differences in the olives constructed based on D.U. Gerstel [6] for Guayule. For the male double circle means the inner allele is hinted in the male due to dominance. Double quarter moon means the two alleles are equally expressed. In the female there is no dominance and all the alleles are equally expressed. When the colour hinted in the male is found in the female the cross is ICO whatever the surrounding colour. When the same colour can contact the cross is IIC. ⊕ ⓪ dissymmetric crosses (six pair wise combinations of genotypes). Self-pollination result is given as **S-S** or **S-F** and discussed in the text.

- year-on-year results may appear quite variable due to temperature variation (consensus in literature, not shown here);
- free pollination for all cultivar ensures an increase in fruit setting;
- pair-wise combination of cultivars may in some case increase the fruit set by 500%;
- in 18% reciprocal fruit set is opposite (±) for pair-wise combinations of cultivars (Table 2).

Villemur et al. [21] have indicated that *Lucques* ovule longevity has the shortest viability during 3 to 4 days compared to 6 to 7 days for other cultivars that may explain no coincidence between blossoming of *Lucques*, which is very early, and its pollinisers. Considering 122 pair-wise combinations of olive cultivars checked by Moutier's team [17–20] and Villemur [21,22], the good coincidence obtained with our model sustains it may run. Most crosses match our model except some exceptions
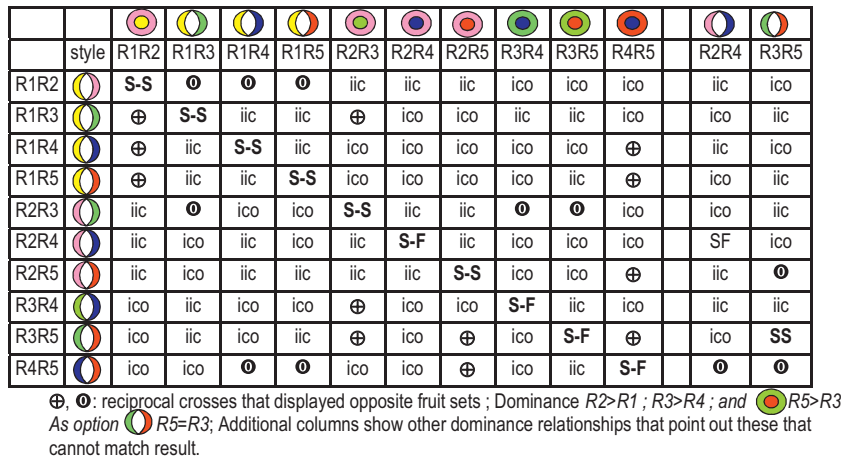
	style	R1R2	R1R3	R1R4	R1R5	R2R3	R2R4	R2R5	R3R4	R3R5	R4R5		R2R4	R3R5
R1R2		S-S	⦿	⦿	⦿	iic	iic	iic	ico	ico	ico		iic	ico
R1R3		⊕	S-S	iic	iic	⊕	ico	ico	iic	iic	ico		ico	iic
R1R4		⊕	iic	S-S	iic	ico	ico	ico	ico	ico	⊕		iic	ico
R1R5		⊕	iic	iic	S-S	ico	ico	ico	ico	iic	⊕		ico	iic
R2R3		iic	⦿	ico	ico	S-S	iic	iic	⦿	⦿	ico		ico	iic
R2R4		iic	ico	iic	ico	iic	S-F	iic	ico	ico	ico		SF	ico
R2R5		iic	ico	iic	iic	iic	iic	S-S	ico	ico	⊕		iic	⦿
R3R4		ico	iic	ico	ico	⊕	ico	ico	S-F	iic	ico		iic	iic
R3R5		ico	iic	ico	iic	⊕	ico	⊕	ico	S-F	⊕		ico	SS
R4R5		ico	ico	⦿	⦿	ico	ico	⊕	ico	iic	S-F		⦿	⦿

⊕, ⦿: reciprocal crosses that displayed opposite fruit sets ; Dominance *R2>R1 ; R3>R4 ; and* ⦿*R5>R3*
As option ◗ *R5=R3*; Additional columns show other dominance relationships that point out these that cannot match result.

Fig. 5. Key to determine inter-incompatibility (0) and inter-compatibility (+) for pair-wise combination of genotypes with parsimony approach up to 5 *S*-allele assuming dominance relationships.

that could be due to self-pollination of the female cultivar that blurred results with the IIC variety. Our prediction agrees with most results in the literature, that suggests examining again the basic data to check whether they have been misinterpreted, or whether it could be justified to move the threshold [17,21]. Many heterogeneous results are given for *Manzanilla* [9,13,15]. We maintained these allele compositions, but more data should be examined.

In most of the situations Vossen [13] has reported self-pollination of the male parent, whereas Moutier [17–21] did not report such results. In fact, Vossen [13] has probably taken into account low S-II (about 0.1–0.2), whereas Moutier's team did not, because they chose 0.3 as threshold. Consequently, our prediction was formerly correct, but anyway we can say that even if self-pollination is possible, cross-pollination will enhance fruit sets. This is due to the position of the threshold for S-II.

Some of those combinations of cultivars that have never been attempted are given as predictions through our model. We indicated for each cross why it failed–presence of the same *S*-allele or why it succeeded–dominance of one allele that enabled to mask a recessive

one, even it was present in the female side and therefore the cross will lead to fruit sets (Fig. 5, Supplementary material, Table S1).

4.1. Same S-alleles from different regions

These allelic compositions show that the olive displays a range of *S*-alleles, but their pair-wise combinations are not wide, leading to difficulties to find adequate pollinisers for each genotype. We have here examined cultivars from different origins: France, Spain, and Greece; moreover, the history of these cultivars rooted them in the Near-East and in North Africa [31], that suggests the *S*-allele set is common to the olive in the whole Mediterranean basin. Crosses of cultivar made with oleaster trees will verify possible gene flow between the wild and the crop that is of importance not only for fruit production, but also for consideration of the evolution of the mating system of both forms.

It is probably useful for olive growers to handle a key to predict whether a cross will be IIC or it will be ICO in a pair-wise combination of cultivars. Here, we do not give all the details, but just a glance on the way to construct the table

Table 2
Summary of *S*-alleles attributed to olive cultivars based on Moutier et al. [17,21,22]; Villemur et al. [18], Musho [9].

	S-S/S-F alleles at the *S*-locus								Best polliniser		
Cultivars		R1	R2	R3	R4	R5	R6	R7			
Manzanilla, Amygdalolia, Cornicabra	S-S	R1	R2							R4R4	R5R5
Bouteillan, VerdaleH, Olivière	S-F		R2		R4				R1R1		R5R5
Picholine, Arbequina	S-S	R1		R3						R4R4	
Cayon	S-F	R1			R4						R5R5
Cailletier	S-F				R4	R5			R1R1		
Salonenque	S-S			R3		R5			R1R1	R4R4	
Grossane		R1				R5				R4R4	
Lucques,Tanche	S-S		R2	R3					R1R1	R4R4	R5R5
Amellau	S-S			R3	R4				R1R1		R5R5
Aglandau	S-S		R2			R5			R1R1	R4R4	
Belgentier [17]			R2				R6		R1R1	R4R4	R5R5

?: Not determined yet.

following the rule that reciprocal cross differences are due to dominance of *S*-alleles in the male only (Table 1). We stressed already that moving the threshold may change the results, but it seems realistic to keep a high threshold for SII (0.3–0.4) to avoid insufficient pollination in case pollination coincides with a rainy period or high temperature, since these are known to decrease olive yields on the whole. Probably, other physiological troubles occur, affecting fruit sets with *Lucques* and *Olivière* that may disturb SSI results and consequently examination of pollen tube growth is required [9,19] for control, but it involves much work. This model will undergo probably some adjustments due to the low numbers of cultivars (#13) with enough crosses and four with a few informative crosses. Data on systematic cultivar pair-wise combinations from other countries could make the model more efficient, for the benefit of the olive community. Moreover, other teams in most countries have plenty of unpublished data and could fulfill diallel tables based on our model. Furthermore, private or rare alleles as R6 and *R5* could be informative on the origin of the cultivars as for *Aglandau*, *Grossane*, *Salonenque* and *Cailletier*, all from Provence.

4.2. Sporophytic self-incompatible prevalent in Oleaceae

The last point deals with the question of SSI in Oleaceae although many reports have favoured GSI. Jasmineaea primitive section of Oleaceae displays heterostyly, an archaic form of SSI [32]. Recently, in the Oleae section, *Phillyrea*, a genus close to *Olea*, Saumitou-Laprade et al. [33] have clearly identified androdioecy due to two *S*-alleles that enable one to maintain male and hermaphrodite trees. Our results suggest that for the olive a SSI mating agrees with works in other genera of the Oleaceae.

In conclusion, we have proposed a model to attribute *S*-alleles to some olive cultivars. Data are not fitting the GSI model. We verified that for almost the whole data the SSI model is accepted. There are still a few inadequacies between data and model, but most predictions are novel and could be verified rapidly to sustain or reject the model. We faced concerns with thresholds to rank cultivars as *S-F* or *S*-I as well as IIC or ICO. It is likely that data from other teams will fulfill our model to the benefit of olive growers. *Cailletier* (called *Taggiasca* in Italy) is as efficient as *Cayon* as polliniser and should not be neglected. Moreover, we suggest to create homozygous pollinisers to ensure efficient pollination, having *R1R1*, *R4R4* and *R5R5* that could each pollinate a wide set of varieties (Table 2). Probably, at the field level some pair-wise combinations of varieties do not occur or are rare due to lack of coincidence of these blossoming. Temperature may shift the blossoming period and thus makes coincidence more or less extended. Anyhow, the main feature in this study stresses that some varieties to be pollinated such as *Tanche* and *Lucques* can accept only *R1* pollen grains from *Cayon*, *Corniale* and *Cailletier*. In the pollen cloud above olive orchards such pollen grains are at low frequency, whereas *R1* pollen grains from *Picholine*, *Manzanilla*, and *Amygdalolia* are certainly much more prevalent. *R4* pollen grains from *Cayon* and *Cailletier* will be also at low frequency in comparison to those (non efficient) from *VerdaleH*,

Bouteillan, and *Arbequina*. If competition may occur on the stigma for pollen germination, it is now clear that olive growers should take into consideration our model to improve fruit setting in the olive, and modify the design of varieties in orchards to ensure better pollination efficiency.

Disclosure of interest

Acknowledgements

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.crvi.2012.07.006.

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