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Rio Grande do Sul Feral Olives May Increase the Species Genetic Variability

Juan M. Caballero[1] and Guajará J. Oliveira[2]

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Abstract

As part of a new wave of expansion in Brazil, olive orchards were planted in a farm later called Cerro dos Olivais (Caçapava do Sul, Rio Grande do Sul) since 2001 to 2006, where frugivorous dark guans have been observed sleeping, resting and eating olive fruit. Olive seedlings were discovered recently under the rows of pines planted close to the mentioned orchards as windbreaks. This *in situ* apparently easy germination suggests that both the passage of the olive endocarps through the birds' intestines and the environment under the pine trees facilitate the germination of the corresponding seeds. The various cross-pollinations that may take place in those olive orchards among the five cultivars growing there may thus increase the olive genetic variability by means of this local feral population. That may give rise to new cultivars, adapted to the special environment of the area by conserving and characterizing the natural progenies developed in the farm.

Pimentel Gomes (1979) dates the arrival of the olive (*Olea europaea*) to Brazil "several centuries earlier in hundreds of the current municipalities" of almost the entire country, even in Manaus, in the heart of Amazonia. Olive trees were planted near the churches, to have bouquets for the Palm Sunday. He talks about olive trees that produced from zero to 200 kg of olives. It also says that the King of Portugal ordered the uprooting of Brazilian olive groves to avoid their competition, although more probably the difficulties in the acclimation of the species when tried at likely the middle of XVIII century were used for discouraging further attempts. Later, in the middle of the 20th century plantations were made in Rio Grande do Sul (farms of 72,000 olive trees in Uruguaiana, 45,000 in Colival, 200,000 in Arroio Grande, with a nursery in Pelotas that produced 500,000 plants per year) and in other states, such as Santa Catarina, Paraná, Sao Paulo, Minas Gerais, Rio de Janeiro.

This private effort, even with some official support, did not give good results. Therefore at the beginning of the 21st century the olive is still rather considered exotic in Brazil while there is a renewed interest in its cultivation. The later called Cerro dos Olivais farm in Caçapava do Sul was the first in Rio Grande do Sul to plant five olive orchards from 2001 to 2006, each with a row of pine trees as protective windbreaks (Fig. 1).

Since they were big enough, those pines have served as resting places for a protected bird, the dark guan (jacuaçu in Portuguese), *Penelope obscura*, of the *Cracidae* family (Fig. 2). Dark guans were already proliferating on the farm due to the planted fruit trees (kiwis, vineyards, orange trees, and pear trees) and the good flora and fauna preservation practices applied during the last 33 years on 50% of the farm.

The purpose of this mini review is to discuss frugivory/seed dispersal in general and that of dark guans, also how the olive seeds have germinated, thus possibly giving rise to wider olive genetic variability due to the dif-

[1] IFAPA Centro Alameda del Obispo, Avda. Menéndez Pidal s/n 14080, Córdoba, Spain, juanm.caballero@yahoo.com

[2] ARGOS (Associaçao Rio-Grandense de Olivicultores), Siqueira Couto 93, Ijuí, RGS, Brazil

Figure 1. Pine trees grow near olive orchards at Cerro dos Olivais farm (Olvania Basso Oliveira)

ferent cross-pollinations determined by the compatibility relationships of the five cultivars planted in Cerro dos Olivais. The risk of olive becoming invasive in the area is also addressed.

Frugivory and seed dispersal

In a study carried out in San Gabriel, about 100 km from Cerro dos Olivais, the dark guan has shown itself as an efficient seed disperser of *Eugenia uniflora*, a native species, and of the exotic species medlar (*Eriobotrya japonica*) and privet (*Ligustrum lucidum*) (Souza 2015).

Work on the Atlantic Forest, a coastal region that includes part of Rio Grande do Sul shows that dark guans eat fruit of *Morus nigra* and *Arcontophenix* sp., considered invasive, as well as a native species; while *Penelope superciliaris* is associated with seeds of *Arcontophenix* spp. (Constantini 2016). On the other hand, analysis of their feces shows that the Northeast jacú (*Penelope jacucaca*) consumes a very varied diet of fruit of different sizes, which varies between areas depending on the available resources (Valtuille 2016).

Many species with fleshy fruits and resistant seeds depend on frugivorous birds for

Figure 2. Dark guans (*Penelope obscura*) at Cerro dos Olivais farm near Caçapava do Sul (Olvania Basso Oliveira)

their natural dispersal and propagation (ornithochory). This is the case for the aforementioned birds and many others in other parts of Brazil, where the *Cracidae* family is one of several that regularly include fruit in their diet (Pizo and Galetti 2010), dispersing undamaged seeds by defecating or regurgitating them. Constantini (2016) showed that 30 of the 39 observed bird species consume fruits or seeds and that 26 of them feed on at least one invasive species. Two of them are large: *Penelope obscura* (68 cm in height and 1770 g in weight, Fig. 2) and *Penelope supeciliaris* (63 cm and 895 g).

In southern Spain the Sardinian Warbler (*Sylvia melanocephala*) and the European Robin (*Erithacus rubecula*) mainly peck the fruit pulp of both cultivated and wild olive trees. The Blackcap (*Sylvia atricapilla*) consumes wild olive fruit mainly by swallowing them, but only pecks the cultivated olives, as the latter are larger. The Song Thrush (*Turdus philomelos*) swallows both kinds of fruit (Rey et al. 1997). Wood pigeons (*Columbus palumba*) eat and disperse cultivated olives in Jaén, the biggest olive area in the world (Perea and Gutiérrez-Galán 2015)

Dispersal of olive endocarps at Cerro dos Olivais

During the harvest of 9- to 14-year-old olive trees dark guans were seen in the olive orchards, in small groups, feeding on olives, using the nearby pine trees for their rest. Since then, a gradual annual increase has been observed. This species is in danger of extinction, so places like this, where hunting is prohibited, are the safest for its survival and proliferation. Surprisingly, during the winter five years later, more precisely in June, many olive seedlings were observed under the pine trees, starting to grow among their fallen leaves and the wild vegetation (Fig. 3). Those seedlings had to come from the germination of the seeds inside the endocarps disseminated by the dark guans through their defecations (Fig. 4).

Figure 3. Olive seedling after germination of seeds dispersed by dark guans (Olvania Basso Oliveira)

Figure 4. Olive endocarps defecated under the pine trees by dark guans (Olvania Basso Oliveira)

Olive germination

If maintained inside their endocarps until harvest, olive seeds having reached their full development undergo a post-maturation process giving rise to a couple of dormancies that inhibit germination, even when placed in good growing conditions. However, it has been observed that if removed from their endocarps 'Arbequina' olive seeds are ready to germinate by the end of August, while those of 'Picual' do so by mid-October at Cordoba, Spain. In both cases it is much earlier than the corresponding fruit are harvested for oil production, especially for 'Arbequina' (Sotomayor-León and Caballero 1989). By those different dates the related embryos have attained maximum dry weight.

The olive seed mechanical dormancy is due to the hard endocarp that is a physical barrier. The endocarp does not inhibit water imbibition, but it cannot be broken by the expanding, germinating seed (Sotomayor-León 1989). In 'Manzanillo' the dormancy is eliminated by chemical scarification (Crisosto and Sutter 1985), but it sometimes damages the inner seeds (Sotomayor-León and Caballero 1990). Sotomayor-León and Caballero (1990) found that this dormancy affects 28% of the endocarps of the five cultivars they studied.

The second type of dormancy related to the post-maturation process mentioned above is called endosperm physiological dormancy. This type of dormancy affected 55% of the naked seeds of five cultivars (also at Cordoba, Spain). This dormancy was eliminated by stratifying seeds at 15 °C under a 16-hour photoperiod for 30 days (Sotomayor-León 1989). Moreover most olive stones of those five cultivars, except those having mechanical dormancy, germinated when stratified properly, but germination were somewhat less under autumnal temperatures in a greenhouse, i.e. 15-20 °C.

Olive pollination

The use of pollinizing cultivars has been traditionally advised in Italy to obtain good olive fruit-set (Scaramuzzi 1976). The apparent success of self-pollination in large, supposedly monovarietal olive masses in Spain may be due to the presence of some trees of other cultivars scattered inside the orchards. Cuevas et al. (2001) verified that cross-pollination improved fruit-set compared to selfing. Paternity studies in monovarietal orchards of 'Picual' and 'Arbequina' also demonstrated that none of the analyzed seeds came from self-fecundation. This has been attributed to the presence of pollen of other cultivars coming from long distances (Díaz et al. 2006). They also showed that in 'Arbequina' monovarietal free-pollinated orchards 'Picual' is its most frequent pollinator, while 'Manzanilla de Sevilla' pollinizes 'Picual' and, to a lesser extent, also 'Arbequina'.

A study of breeding progenies showed that the crosses 'Arbequina' x 'Picual', 'Arbequina' x 'Manzanilla de Sevilla', 'Manzanilla de Sevilla' x 'Koroneiki' and their reciprocals were compatible, but not the 'Arbequina' x 'Koroneiki' nor its reciprocal (Díaz et al. 2007). On the other hand, a review on olive pollination reports that 'Arbequina', 'Picual' and 'Manzanilla de Sevilla' fruit do not come from self-pollination, but they do in 'Koroneiki', so this cultivar is one of the very few self-compatible olive cultivars (Rodríguez-Castillo et al. 2009). Moreover, 'Arbequina' does pollinate 'Arbosana', but 'Picual' does not, whereas 'Koroneiki' is pollinated by 'Picual' and 'Manzanilla de Sevilla'.

Olive genetic diversity: wild olives, cultivars and feral forms

Olea and *Ligustroides* are the two sections of the subgenus *Olea*, one of the three that make up the genus *Olea*. Olive (*Olea europaea* L.), is a species of great world importance, composed of six subspecies, the first of which is the European olive (*Olea europaea* L., ssp. e*uropaea*), formed by the wild olive trees (var. *sylvestris*) and those cultivated (var. *europaea*). In general the fruits of wild olive trees are smaller than those of cultivated trees (less than 1 cm long versus 2-4 cm) and have thin versus thick mesocarps. The term wild olive also encompasses feral olive trees (Green, 2002), as they come from the germination of cultivars' seeds produced by free-pollination among them, with wild olive trees or with other ferals (Angiolillo et al. 1999).

Seed propagation, inherent to wild olive propagation, is the main source of olive genetic variability accumulated through millennia in the Mediterranean Basin, verified in wild (Belaj et al. 2011), cultivated (Caballero and Del Río 2005) and feral olive trees (Angiolillo et al. 1999; Guerin et al. 2000). Olive cultivated varieties (cultivars) represent an important sample of the olive variability, fixed by cloning each selection among wild olives by vegetative propagation, either by rooting large propagules or by grafting on seedlings (Caballero and Del Río 2017). But olive trees of the first selections have been successively crossed with those of newer cultivars and/or wild olive trees in the same area. There was also movement of cultivated materials, mainly to the West from the Northeast of the Fertile Crescent (near to the Syrian-Turkish border), where the most important olive domestication event took place (Besnard et al. 2013). On its turn the already cultivated material exported from that area would give rise to secondary domestication by crossing with local wild trees across the Mediteraanean Basin.

Another study (Díez et al. 2015) showed that in the Mediterranean Basin the genetic diversity of 96 wild olive trees makes it possible to distinguish a Western group (48 from southern Spain and 10 from Sardinia, Italy) and an Eastern one (38 from Israel). It also assigns the 289 cultivars studied to three genetic groups. The Q1 or Occidental group contains accessions mainly from southern Spain and is closely associated with a putatively feral individual from the Eastern Mediterranean Basin, in which today's wild olive trees are rather feral. Q2 is mainly made up of accessions from the Central Mediterranean, with a notable genetic contribution from wild olive trees similar to those still existing in Spain and Sardinia. Q3 is mainly made up of cultivars from the Eastern Mediterranean. In addition, Q2 cultivars may be the result of an intense admixture between cultivated materials coming from the Eastern part and local wild germplasm or, more probably according to the authors, they come from local domestications later admixed with materials brought by migratory currents from the East.

The search for big and oily fruits must have been the main objective of the mentioned successive selections of wild trees. In that subspecies fruit weight, pulp/stone ratio and fruit dry matter oil content are 0.46 g, 2.07 and 20.3%, respectively (Belaj et al. 2011), but 4.0 g, 7.0 and 43.8% in a world germplasm bank (Del Río et al. 2005) and 2.9 g, 6.4, 43.7% in a breeding progeny (León et al. 2004). Fruit weight of the aforementioned sample of wild olive trees varies from 0.15 to 1.26 g and their fruit dry matter oil content from 7.8 to 33.8%, although some of them may be of feral origin. The collections of the ten most important olive-growing countries of the Mediterranean Basin counted 1304 cultivars (Caballero and Del Río 1999). In the absence of others, the results of the Spanish varietal surveys made it possible to estimate that at least 1,500 cultivars were being grown in the world (Caballero et al. 2006).

Since 1956 to 1962 China imported up to six batches of olive trees, cuttings, and endocarps of various cultivars from Albania and Crimea (Yu, personal communication). New cultivars have been selected from 25,780 seedlings originating from those imported endocarps, whose male parents are unknown (Ying et al. 1980; Yu 2012). This new material is undoubtedly of feral origin, since it comes from endocarps of cultivated varieties sent from Europe to China.

Spain introduced the olive in America at the beginning of the 16th century, along with wheat and grapes, thus transferring the typical triad of the Mediterranean Basin. It is estimated that the olive expansion was by rooting hardwood cuttings, but also by seed germination and subsequent budding or grafting (Caballero 2013). Today the latter system is still used in various American countries, therefore it is quite sure that in almost 500 years new genetic variability has emerged in America by means of feral trees produced by unwatched graft losses, either in nurseries or in the field. Thus, there must be new genotypes, some of which may have given rise to new cultivars. A visit to the collections of Argentina, Brazil and Chile and information from California identified 51 accessions, with some repeated names, without counting introductions since the 19th century (Caballero 1995 report to FAO).

In Chile a morphological characterization of the olive genetic variability describes 19 different varietal denominations from 37 marked olive trees, with five synonyms (Tapia C. et al. 2001). Fifteen cultivars were identified, six of them present in the World Olive Germplasm Bank of Córdoba (Spain): five of them from Spain, Italy and Greece, the sixth ('Azapa') was received from Chile. In a similar work in Catamarca, Argentina (Cólica 2008) 111 olive trees were marked, corresponding to 66 different names, from which 62 cultivars were identified, of which 16 are in the aforementioned World Bank. The main of these cultivars, 'Arauco', received from Argentina, is identical to 'Azapa' (Caballero and Del Río 2005, Caballero 2013).

'Azapa' is also called 'Sevillana' in Chile, where it was observed to differ genetically from 20th centennial olive trees, also different among them: ten from each one of Azapa and Huasco valleys (Contreras et al. 2018). They suggest that 'Azapa' could have been taken to America quite early, in the 16th century. 'Azapa' is also grown in Perú with other names (Tapia, personal communication). But it could also be that its origin is a feral olive tree produced *in situ*, when a graft was lost somewhere at the very beginning of the colonization. Grafting, along with planting hardwood cuttings, was used in the area since the 16th century (Inca Garcilaso de la Vega 1617). This cultivar is widely spread in those three countries although with different names.

Mekuria et al. (2002) studied feral olive trees found in an isolated site on Kangaroo Island (South Australia). Eight of the 24 feral trees studied are genetically associated with four of the nine olive trees planted in 1900, and the other 16 with one of them. The original trees could not be identified. The derived feral olive trees are considered part of a spontaneous progeny of those original five, from which they were separated by 100 to 450 m, so animals or birds had to intervene in the dispersal of the germinated seeds. In Australia the potential of feral olives as source of new cultivars was early recognized and new selections were made, 'Hardy's Mammoth' being the most successful (Hardy 1901, 1902, cited by Spennemann and Allen 2000). Soleri et al. (2010) found eight feral trees in a sample of 81 out of 453 in two olive groves on Santa Cruz Island, 40 km off Santa Barbara coast of California, where 'Mission' and 'Redding Picholine' trees had been planted in 1876-1877.

So since the beginning of olive tree domestication, feral forms have also participated in the aforementioned flow and exchange of genes, increasing variability. Indeed, that observed among the oils of 231 other feral olive trees in Australia shows an oleic acid content range of 40.9-87.7% (Guerin et al. 2000), while in a group of 74 cultivars from the World Germplasm Bank of Córdoba it is of 56.1-78.6%, respectively (Uceda et al. 2005). The range of the olive dry matter oil content of these feral olive trees is 6.4-66.8% while that of another group of 61 cultivars from the aforementioned Germplasm Bank is 32.2-59.3% (Del Río et al. 2005).

Discussion and Conclusions

The ability of the dark guan (*Penelope obscura*) to eat and disperse big fruits (Constantini 2016, Souza 2015) suggests that it can also ingest tiny olive fruits upon their appearance in Cerro dos Olivais. Similar to the *Penelope jacucaca* (Valtuille 2016), passage of olive endocarps through its gut may scarify them (Fig. 4), thus facilitating germination (Fig. 3) by eliminating their almost stony consistency (Crisosto and Sutter 1985). Besides that, in Spain *Turdus philomelos*, a bird much smaller than *Penelope obscura* eats both wild and cultivated olive fruit (Rey 1997) and wood pigeon (*Columbus palumba*), a not big bird either, also eats cultivated fruit (Perea and Gutiérrez-Galán 2015)

As the olive fruits gradually change color from dark green to yellow-green, violet and black during maturation, dark guans may eat them quite early, when seeds are ready to germinate before the post-maturation phase (Sotomayor-León and Caballero 1989). In that case the seeds may start germinating as soon as the endocarps are defecated by the guans under the pine trees. When fruits are eaten later, when black, the seeds inside the endocarps may undergo the post-maturation process through a kind of stratification caused by the high humidity under the fallen leaves and the wild vegetation under the pine trees. The local fall-winter temperatures are quite similar to those explained above (Sotomayor-León 1989) and can satisfy requirements for olive endosperm physiological dormancy (Clima característico em Caçapava do Sul, Brazil durante o ano - Weather Spark 13-6-2020.html).

Genotypes of the open pollinated feral olive trees of Cerro dos Olivais were undoubtedly the product of the compatible cross-pollinations between the planted cultivars: 'Arbequina', 'Arbosana', 'Koroneiki', 'Picual' and 'Manzanilla de Sevilla'.

Therefore most of the reported feral seedlings will be progeny of 'Arbequina' as it is the most abundant cultivar on the farm, with approximately 70% of trees, obtained with pollen from 'Picual', but also from 'Manzanilla de Sevilla'. There may also be self-pollinated 'Koroneiki' progeny. Depending on the simultaneity of the compatible cultivars' blooming times, possibly affected by the local climate, there may also be seedlings from pollinations of 'Arbosana', 'Picual' and 'Manzanilla de Sevilla' by 'Arbequina', 'Koroneiki' pollinated by 'Picual' and 'Manzanilla de Sevilla ', from' Picual' by 'Manzanilla de Sevilla', and 'Manzanilla de Sevilla' by 'Koroneiki'.

This potential wide genetic variability of the feral seedlings germinating under the pine trees, due to the frugivorous activity of the dark guans, will likely be also affected by the possible different preferences of these birds for the olives' sizes, colors and flavors. Olive size also varies from year to year in the same cultivar because of crop load, thus possibly adding more variability to the seedlings already obtained and those which could be produced in the future.

The aforementioned antecedents from China, America and Australia allow thinking about conserving, selecting and characterizing this natural progeny, from which new cultivars could arise. Their interaction with various cultivation environments of the olive world should also be studied, starting with the special one of Rio Grande do Sul, as they will be more adapted to their place of origin. The climate is quite humid year round, where average summer precipitation ranges from 111 mm in February to a maximum of 176 mm in March).

Care should however be taken to avoid the risk of feral olives becoming invasive in the area, as already noted in Australia, where several measures to control them were advised (Spennemann and Allen 2000). In California 14,000 feral olives had been removed by the Channel Island National Park (Soleri et al. 2010). In Spain wood pigeons (*Columbus palumba*) are dispersing cultivated olive trees up to 7.4 km inside a protected vegetation area, thus also risking olive feralisation (Perea and Gutiérrez-Galán 2015)

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