Breeding System Evaluation of Four *Mangifera indica* Linnaeus (Anacardiaceae) Cultivars in Puerto Rico[1]

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Abstract: Germplasm in the form of living collections can make important contributions to the understanding of how processes that occur at the pollination stage influence reproduction and plant yields. Using artificial pollination experiments this study evaluated the degree of self-compatibility, dependence of their reproductive systems on flower visitors to set fruit and potential effect of pollen type (self *vs* cross *vs* open pollination) of four commercial cultivars ('Julie', 'Keitt', 'Kent' and 'Tommy Atkins') at the Juana Díaz Experimental Station (JDES) in Puerto Rico. Open natural pollinations tended to produce more fruit and yielded seeds with faster development times than artificial pollinations in three of the four cultivars ('Julie', 'Tommy Atkins' and 'Keitt'). Also resulted in faster seed germination in two cultivars ('Julie' and 'Tommy Atkins'). The combined results may suggest that not only animal mediated pollination is needed for these cultivars but that this mode of pollination also results in improved yields, fruit, and seed traits. The importance of studying the breeding system of all living germplasm and for *M. indica,* a fruit crop with variable breeding systems throughout its distribution results reiterate the importance of local pollinators and the need to evaluate the circumstances by which pollen donors may influence fruit characteristics in this crop.

Key Words: mango, germplasm, pollination systems, flowers, fruit performance, Puerto Rico

Introduction

Protection and conservation of germplasm material from agricultural crop is essential to global food security and has become a priority given the loss of biological diversity worldwide (Thrupp 2000, Anonymous 2018). A crop's germplasm constitutes the collective catalogued genetic pool available (wild and domesticated) for crop development and improvement (Orton 2020). Germplasm provide the raw material to develop new crops and as such it is the foundation for current and future agricultural development (Peefers and Calwey 1988, Nwachukwu et al. 2016). Many ex-situ germplasms collections of

agricultural crops are maintained as seeds and tissue but also as field living collections of old, current, and wild varieties of crops. With the purpose to study the manage, use and conserve their genetic diversity (National Research Council 1991). While seeds are the primary collection for herbaceous plants, woody perennial crops rely heavily on living collections because their longer life history cycles and different seed characteristics (e.g., larger, heavier, fleshier) preclude many of these cultivars from relying on seed-based genetic banks (Migicovsky et al. 2019). This is particularly true for tropical fruits whose fleshy fruits with high moisture content prevent seed storage and transport through conventional cold dry storage methods (Bonner 1990; Tweddle et al. 2003, Li and Pritchard 2009, Walters et al. 2013). Living collections of woody perennial crops although expensive to maintain, important opportunities to conduct biological research to support conservation and management of agricultural diversity (Migicovsky et al. 2019).

Basic biological information of agricultural plant species such as its flowering biology, mating systems, seed production and viability are important aspects of long-term effective management and future use of agricultural germplasms (Engels and Visser 2003). For example, determining seed vigor, in addition to germination percentage, could provide the germplasm's curator with early indications of viability decline in the genetic stock (Bewley and Black 1994). Knowledge of the mode of reproduction of a crop and how it reproduces naturally would be essential to evaluate which possible methods of reproduction can be employed for artificial breeding, for crop improvement and to predict a cultivars' behavior under field conditions (Fryxeli 1957). A crop's breeding system will also influence the level of genetic variability present in the crop population (Camarena Mayta et al. 2014).

The role of animal pollinators in agriculture is often cited as an important and risk ecosystem service (Klein et al. 2007). For tropical crop species, an estimated 70% of them, have cultivars where production is improved by animal pollination (Roubik 1995). Even when during the process of domestication, self-pollination has often been a favored trait, many crop species are predominantly cross-pollinated (Halloran and Luckett 1994). Woody perennial crop species tend to be predominantly outcrossing (other mechanisms of cross) (Migicovsky et al. 2019) and would need to rely on animal pollination for reproduction. When crop plants may exhibit self-compatibility seed quality may be enhanced by outcrossing (Richards 2001). However, without reliable information on the breeding system and the mechanics of natural pollination, identifying the factors that lead to failure or success in crops of interest will be difficult.

Mangifera indica (mango), originally from Asia, has had a long and complex history of domestication with over 1000 cultivars developed and established throughout the worlds' tropics (Litz 2009, Warschefsky and Westberg 2019). It has been claimed that almost all known cultivars have originated from the selection of chance seedlings from natural cross-pollinations (Iyer and Schenell 2009). Yet, the literature suggests that the breeding and mating systems of this tropical fruit crop is highly diverse among cultivars. For example, some mango cultivars such as Dasheri and Ataúlfo are self-incompatible (Singh et al. 1962, Davenport 2009) and it is assumed that insects play an essential role in their pollination even if not understood (Anderson et al. 1982). In contrast, cultivars such as Sensation, Osteen, 'Keitt' and 'Kent' reportedly show capacity for self-pollination (Dijkman and Soule 1951; Pérez et al. 2016). However, in the case of the Osteen, 'Keitt' and 'Kent' cultivars, molecular tests show a higher percentage of progeny resulting from outcrossing mating relative to selfing (Pérez et al. 2016).It has even been suggested that wind and the falling of grains by gravity could facilitate pollination in some mango cultivars, but experimental data has shown that even, if possible, pollination success is very low with this mechanism relative to cross-pollination (Popenoe 1917, Maheshwari 1934, Mallik 1957, Singh 1997, Singh and Sharma 1972). One study suggests that cross-pollination may lead to higher fruits sets than self-pollinations, but this may vary among cultivars (Paull and Duarte 2011). The data available for *M. indica* cultivars from the literature suggest that their mode of reproductions is understudied, and incomplete given the large number of cultivars available.

There are many crops of local economic importance whose yield or quality may be enhanced by good pollinator activity (Richards 2001). Klein et al. (2007) found inadequate information is available on the pollination biology and pollinator requirements of many crops. In the case of mango, depending on the cultivar and geographical location, data reflects a potentially large variability among cultivars in their pollination biology, natural pollinators, and breeding systems (Pérez et al. 2016, Sharma and Singh 1970, Dag et al. 2000). This study aimed to characterize the breeding system of these four commercial cultivars of *M. indica* ('Julie', 'Keitt, 'Kent' and 'Tommy Atkins') grown at germplasm had the Juana Díaz Experimental Station (JDES) in Puerto Rico to evaluate their degree of self-compatibility, the dependence of their reproductive systems on flower visitors to set fruit and the potential effect of pollen type (self vs cross vs open pollination) on their seed and fruit performance. Of these four cultivars three of them come from Florida material ('Keitt', 'Kent', 'Tommy Atkins') being part of a germplasm of 84 mango cultivars planted in 1962. In this study, we will investigate the following question: 1) How dependent is the breeding

system of these cultivars on animal pollination? 2) What is the effect of mating type (self vs cross-pollination) on reproductive success, fruit and seed traits of these cultivars? To answer these questions, the study evaluated two hypotheses. Firstly, we expected different cultivars to show different breeding systems, where some cultivars will necessarily depend on pollinators and others will not. Second, we expected that the different mating types would show differences in fruit set, fruit and seed traits under the assumption that cross-pollination may influence the genetic variation and phenotypic expression of the developing embryo and therefore the quality of fruits and seeds. There are many crops of local economic importance whose yield or quality may be enhanced by good pollinator activity (Richards 2001). Klein et al. (2007) found inadequate information is available on the pollination biology and pollinator requirements of many crops. In the case of mango, depending on the cultivar and geographical location, data reflects a potentially large variability among cultivars in their pollination biology, natural pollinators, and breeding systems (Pérez et al. 2016; Sharma and Singh 1970, Dag et al. 2000). To evaluate these hypotheses, we conducted pollination experiments to elucidate the reproductive success (fruit production, fruit development and seed germination) of different mating (self vs cross vs open pollination). This work builds of plant reproductive ecology theory to provide important information to support germplasm conservation in *M. indica*, an economically important crop in the world's tropics.

Methods
Study Sites
    This study was conducted on the mango orchard of the Agricultural Experimental Station (JDES)located in Juana Díaz in the South of Puerto Rico (180 01'N, 66 031'W, 22.25 msl). This research center has an area of 111.23 ha, recorded average monthly temperature range between 22°C to 33°C and monthly average rainfall of 977.1 mm (Harmsen et al. 2014). The site is located in a subtropical dry forest life zone which makes it an ideal area to maintain different fruit germplasms (Ewel and Whitmore 1973). One of the main germplasmic materials brought to JDES is the Mangifera indica cultivar collection. Today's collection maintains 84 of the 105 mangos cultivars that were initially planted in 1968. The total mango germplasm occupies two sectors of JDES covering 14.16 ha (Figure 1). In Juana Díaz Agricultural Experimental Station, all cultivars are managed equally, the germplasm have been given the same management to all trees, the distance between trees in the germplasm was a 4.6 m.by 7.62 m. between rows. Weekly irrigation, fertilizer twice per year and monthly weed management is provided.
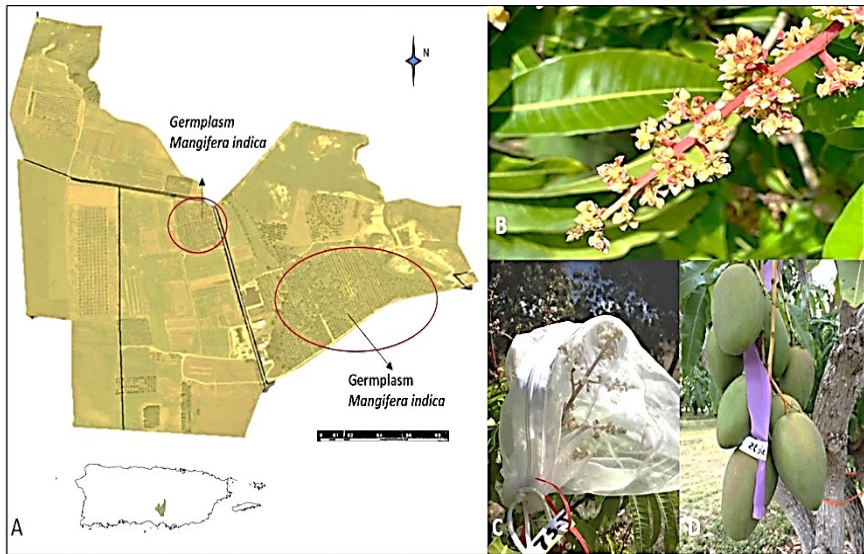
Figure 1. A. Location of the germplasm of Mangifera indica in the Agricultural Experiment Station of Juana Díaz. B. Flower of 'Julie', C. Self -treatment of 'Julie', D. Fruits of control treatment in 'Julie'.

Study System

Mangifera indica L. is a perennial tree belonging to the family Anacardiaceae. The flowers of the mango are found in a panicle that is composed of a main axis with several secondary axes branched into tertiary and sometimes quaternary axes (Coetzer et al. 1995, Goguey 1997). Plants produce minute flowers (5-10 mm diameter), with yellow nectar discs and can be hermaphrodite and male both of which have stamens (Kostermans 2012). Hermaphrodite flowers have a globose ovary with a single stigma (Ramirez and Davenport 2016). The mango pollen is variable size (25 to 45μm long) and has an oval shape when dry and is sphere-shaped when hydrated (Ramirez and Davenport 2016, Popenoe 1917). The four mango cultivars studied are Keitt, Kent, Tommy Atkins, and Julie. There are some noticeable differences among the four varieties selected for the study. Keitt which is the most sown cultivar in Puerto Rico has a medium to moderate tree height (9.1 m to 40 m), an open canopy and a fruit size that ranges from 13 to 15 cm long (Campbell 1992). The Kent cultivar is the preferred cultivar in Latin America and considered a tall tree can reach 40m that produces fruits with sizes ranging from 11 to13 cm long (Campbell 1992). The Tommy Atkins cultivar, the most preferred for the color of its fruit (skin orange-yellow, crimson, or dark red blush), has trees with a dense and round canopy and the fruit can measure 12-14.5cm. long (Campbell

1992). Julie is the cultivar with the smallest tree height reaching up to 3m. This cultivar is believed to have come from Jamaica Caribbean (Morton 1987). For all varieties, flowering occurs mostly once a year starting in January (or end of December) with the flowering season lasting five to six weeks (Cabrera-Asencio, unpublished data). In Juana Díaz Agricultural Experimental Station, all cultivars are managed equally, the germplasm have been given the same management to all trees. Providing weekly irrigation, twice per year fertilizer and monthly weed management.

Pollination experiments

    From January to April 2019, 10 trees per cultivar were selected and five different pollination treatments commonly used to evaluate a species breeding system (control, apomixis, autogamy, self and cross pollination, Eckert 2010) were conducted on each tree. The control treatment consisted of non-manipulated flowers sitting on open inflorescences (natural pollination). In the remaining treatments, flowers were manipulated, and their respective inflorescences were covered with an organza bag. The apomixis treatment consisted of emasculated flowers (asexual fruit production). The autogamy treatment consisted of unmanipulated flowers covered with an organza bag (autonomous pollination). The last two treatments consisted of manual pollinations (self-pollination and cross pollination) which were performed when the pistil and anther of selected mature flowers were put in contact with each other: In the self-pollination treatment flowers were pollinated using pollen from the same tree and the same inflorescences; In the cross-pollination treatment flowers were pollinated with pollen from another tree (at least nine meters away) and inflorescences. For all treatments with the exception of the control, inflorescences were covered with organza bags before the opening of tagged flowers to avoid the presence of flower visitors before the experiment began. The organza bags were kept during five weeks, until the flowers either senesced or else formed fruits. Each treatment contained 10 replicates for a total of 500 pollinated flowers distributed across all treatments. Pollinations were performed weekly during the first four weeks of flowering and then checked weekly to record fruit development time, % fruit initiation (number of fruits initiated/flowers observed x 100) and final fruit production or % fruit set (number of fruits matured/flowers observed x 100). Matured fruits from all treatments were collected to record their weight and determine their seed germination success (see below).

Seed germination

 To determine the germination rate of seeds, the seeds were extracted from the fruit and the endocarp removed in order to examine the embryo. Once the embryo was removed, they were placed in trays where they were covered with wet paper towels to keep them moist, and they were kept in darkness at a constant temperature of 30°C in an environmental test chamber (Panasonic MODEL MLR-352). For each tree, the percent seed germination was determined as the number of seeds germinated / the number of seeds available for that treatment.

Statistical analyses

 Statistical analyses were used to evaluate differences among the other sets of treatments (self-pollination vs cross-pollination treatments, control vs average of self and cross-pollination treatments, control vs cross-pollination) in the following dependent variables for each cultivar: fruit set (%), fruit development time (days), fruit weight, seed germination (%) and seed germination (days). The apomixis and autogamy treatments did not produce fruits and were excluded from the analysis. For the variables fruit set (%), seed germination (%) and seed germination (days), we used paired t-tests to evaluate differences between paired treatments in cultivars Julie, Kent, and Tommy Atkins. For the cultivar Keitt we used inference with Wilcoxon test, because the data was nonparametric. To analyze fruit development time (days) and fruit weight, we used the values for the differences between paired treatments (i.e., self- vs cross-pollination and control vs. average of self- and cross-pollination) and then used Two-way ANOVA to evaluate if there were statistical differences among cultivars in the mean deviation values between treatments. When the data was non-parametric, we used Kruskal Wallis Test.

 We constructed a reproductive index (IR) using the following formula for each treatment: IR = (% fruit set/100) x (cultivar's average development time of all treatments/minimum development time of treatment) x (average weight fruit) x (% seed germination/100) x (cultivar's average days to first germination/ minimum number of days to first seed germination). We used a Two-way ANOVA to evaluate if there were statistical differences in IR between cultivars and pollination treatments.

Results

Fruit traits

 Fruit set. Out of the five pollination treatments two treatments, autogamy and apomixis failed to produce fruits. For these treatments, flowers senesced 14 days after treatment for all cultivars. We found no significant differences in

the proportion of flowers that set fruit between the self and the cross-pollination treatments regardless of cultivar (Figures 2A, 2C, 2E, 2G). However, for three cultivars (i.e., 'Julie', 'Keitt', 'Tommy Atkins'), flowers in the control treatment (i.e., open pollinations) produced proportionally more fruit on average than the combined average for the self- and the cross-pollination treatments (Figures 2B, 2D, 2H).'Julie' and 'Tommy Atkins' had the highest average percent of fruit set with 78% (±14.92) and 78% (±14.90) respectively compared to an average fruit set for the combination of self and cross pollination treatments (Figures 2B, 2H). Following the cultivar 'Keitt' had and a moderate average fruit set with 55% (±19.44, Figure 2D).

Fruit development time. ANOVA analyses yielded significant differences among cultivars and pollination treatments in fruit development time as well as a significant cultivar*treatment interaction in this variable (Table 1A). On average, fruits for the 'Keitt' and 'Kent' cultivars had longer fruit development times than fruits from the 'Julie' and 'Tommy Atkin' cultivars regardless of pollination treatment (Figure 3). Fruits from the 'Julie' and 'Tommy Atkin' cultivars developed on average 16 days faster than fruits from the 'Keitt' and 'Kent' cultivars (average number of days(d) ± SE: 'Julie' = 117d ± 1.52, 'Tommy Atkins' = 117d±1.44, 'Keitt'=136d±1.58, 'Kent'=131d±1.52). Significant differences in fruit development time among pollination treatments were only evident for the 'Julie' and 'Tommy Atkins' cultivar but they did not express equally. In the 'Julie' cultivar, control pollinations resulted in fruits that developed on average 9d (111.81±1.32) faster than those from self and cross pollinations with no significant differences in fruit development time between self- and cross-pollinations (Figure 3). In contrast, in the 'Tommy Atkins' cultivar, fruits from the self and control treatments developed 1-2 days faster than those from the cross-pollination treatments with no significant differences between those two (averages ± SE: Control: 117.2d ± 1.29, Self: 118.0d ± 1.58, Cross: 116.8d ± 1.46).
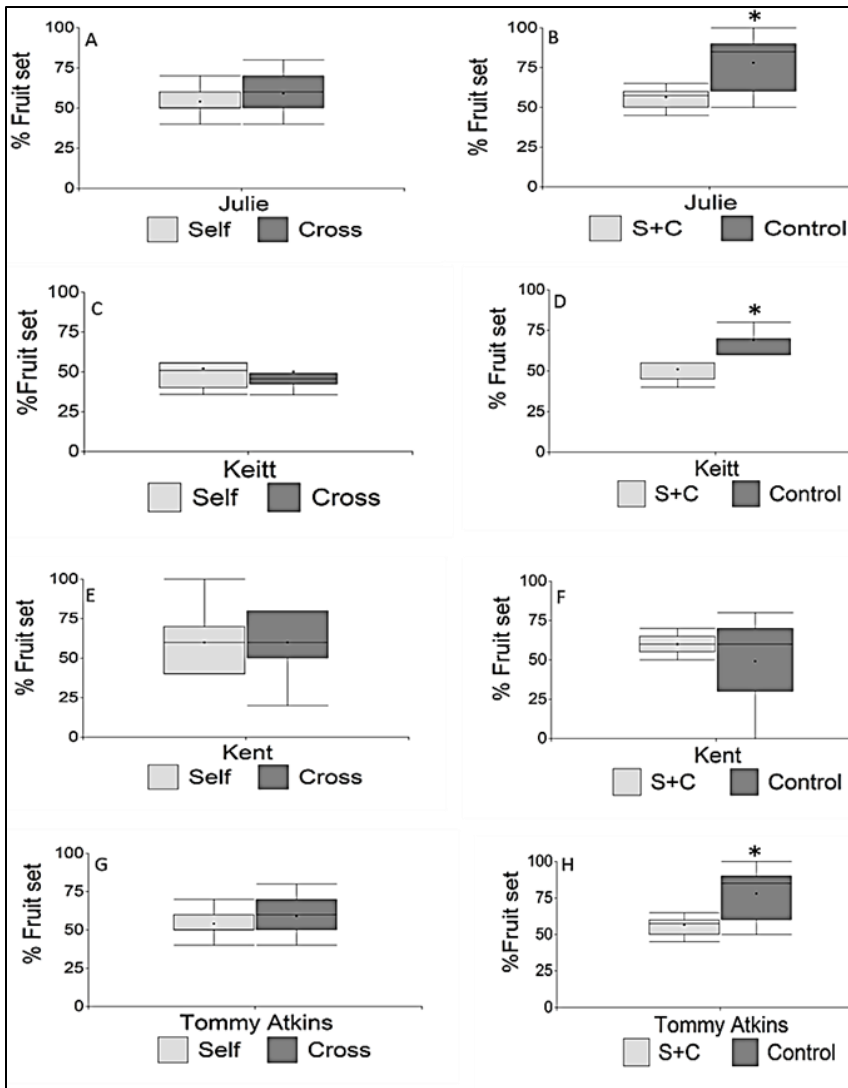
Figure 2. Comparison of percent fruit set between pairs of pollination treatments: self- vs cross-pollination and average of self and cross pollination (S+C) vs pollination of open flowers (control) in four different cultivars of *M. indica* ('Julie', 'Keitt', 'Kent', 'Tommy Atkins'). Analyses represent paired-t tests for all cultivars except for comparisons for the 'Keitt' cultivar which were with Wilcoxon tests as data did not meet all assumptions for parametric tests. The significant values were indicated by "*" and represent $\alpha = 0.05$.

Table 1. Model two way-ANOVA to evaluate differences in: A. Days of fruit development to ripe and fruit weight in different breeding systems in four cultivars.

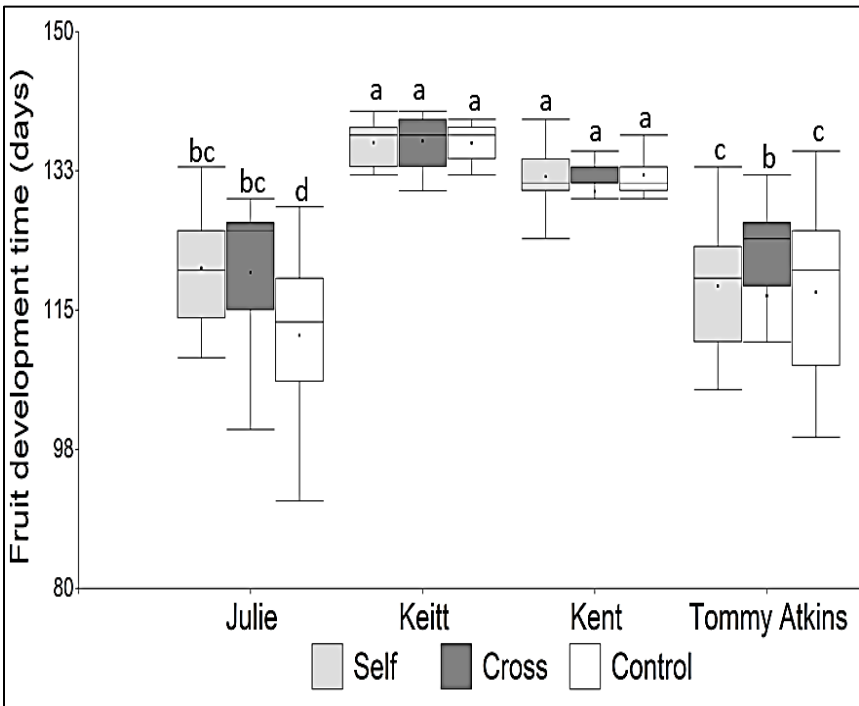| A. ANOVA	Days of fruit development		
Model		*F*	*p*
Cultivar	323.25		0.0001
Treatment	15.73		0.0001
Cultivar * Treatment	7.79		0.0001
B. ANOVA	Fruit weight		
Model		*F*	*p*
Cultivar	167.47		0.0001
Treatment	10		0.0001
Cultivar * Treatment	2.16		0.0442

Figure 3. Variation in the duration of fruit development as a function of *M. indica* cultivar ('Keitt', 'Kent', and 'Tommy Atkins') and pollination treatment (self, cross, control). Different letters indicate significant differences (at α=0.05) following post hoc tests following Two-way ANOVA analyses (Table 1-A).

Fruit weight. Results from two-way ANOVA detected significant effects of cultivar, pollination treatment and their interaction on fruit weight (Table 1B). Overall, 'Julie' produced lighter fruits than the other cultivars regardless of treatment with fruits from the other cultivars ('Kent', 'Keitt', and 'Tommy Atkins') weighing 140g more on average. Meanwhile, cross pollinations tended to yield slightly heavier fruits than self and control pollinations but only in the 'Keitt' and 'Tommy Atkins' cultivars (Figure 4). Fruits from cross pollinations were on average 58g heavier than controls in the 'Tommy Atkins' cultivar and 51g heavier than controls in the 'Keitt'. In both of these cultivars, cross pollinations tended to be heavier than self-pollinations, but that tendency was not significant (Figure 4).
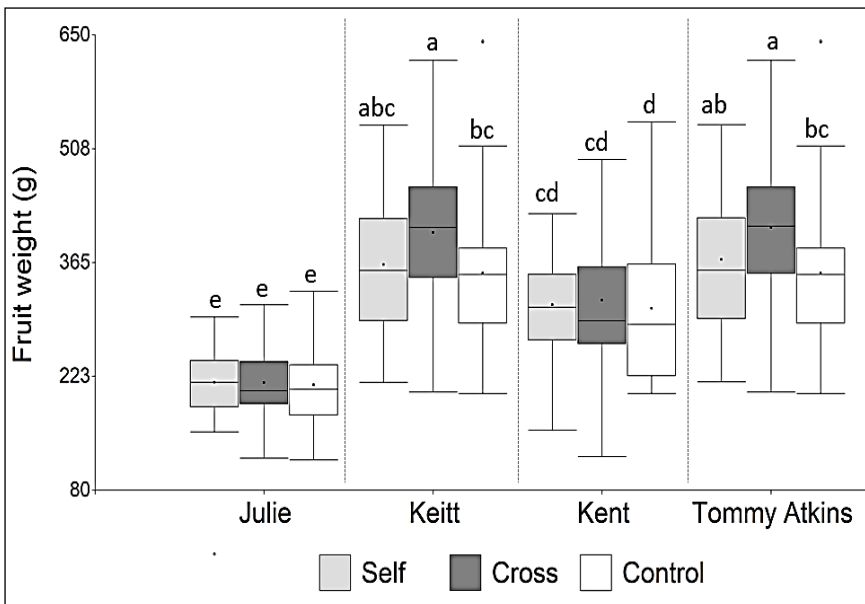

Figure 4. Differences in average fruit weight among cultivars ('Julie', 'Keitt', 'Kent', and 'Tommy Atkins') and pollination treatments (self, cross, control) in *M. indica*. Different letters indicate significant differences at α =0.05 as determined by post hoc analyses following Two-way ANOVA (Table 1-B).

Seed traits

Seed germination. The average percentage of seed germination ranged from 32% to 80% (Figure 5). None of the cultivars exhibited significant differences between self and cross-pollination treatments in the percentage of germinated seeds (Figures 5A, 5C, 5E, 5G). However, for three of the cultivars ('Julie', 'Keitt', and 'Tommy Atkins'), the control (open) pollination treatment had

significantly higher germination percentages than the combined average for the self and cross pollination treatments (Figures 5B, 5D, 5H).
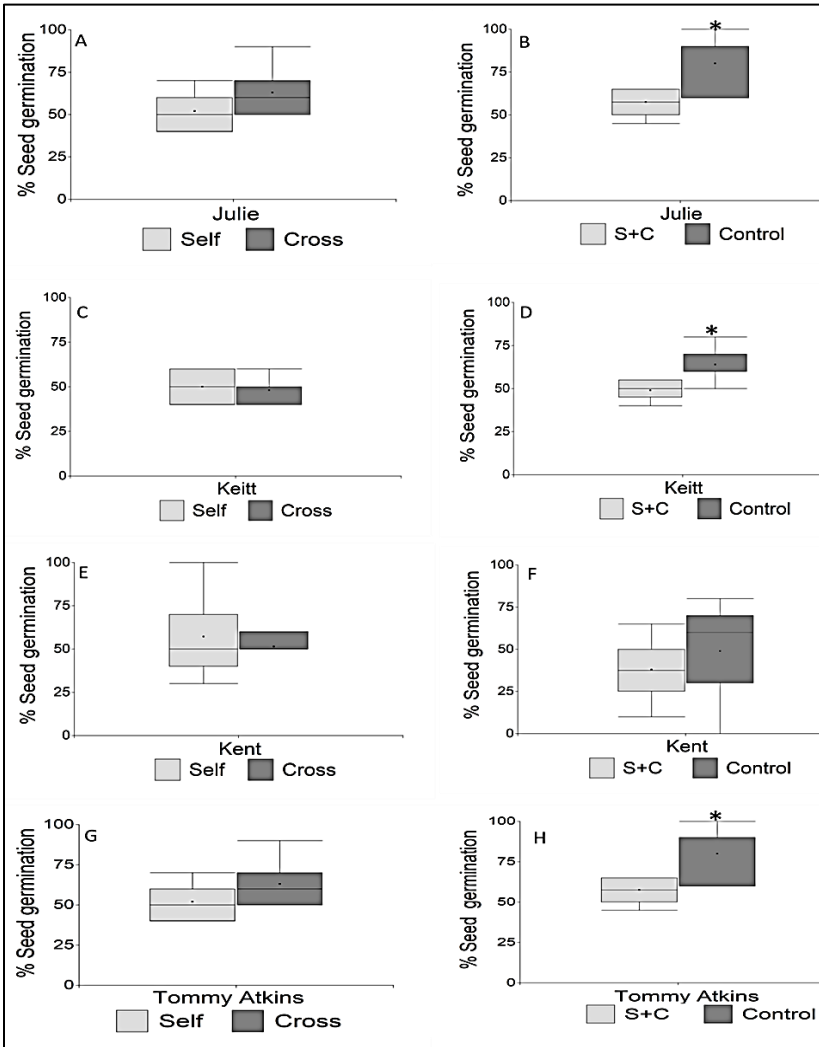

Figure 5. Differences in percent seed germination between pollination treatment pairs: self-vs cross-pollination and average of self and cross pollination (S+C) vs pollination of open flowers (control) in four different cultivars of *M. indica* ('Julie', 'Keitt', 'Kent', and 'Tommy Atkins').   Analyses represent paired-t tests for all cultivars except for comparisons for the 'Keitt' cultivar which were with Wilcoxon tests as data did not meet all assumptions for parametric tests. The significant values were indicated by "*" and represent $\alpha = 0.05$.

In the 'Julie' cultivar there was a 23% difference between the average percentage for seed germination of the control treatment and the combined hand pollination treatments while for the 'Keitt', and 'Tommy Atkins' cultivar that difference was 15% and 23% respectively.

Seed germination days. On average seeds took 3.80 to 4.07 days under a control environment (Figure 6). There were no differences between pollination treatments in the days it took seeds to germinate except for the 'Tommy Atkins' cultivar in which seeds from control (open) pollinations developed slightly faster than seeds from hand pollinations (open: 4 ±0.12d SD, self +cross: 3.86 (±0.12d SD, Figure 6H).

The reproduction index (IR) which combined all reproductive characteristics was significantly different among cultivars with Tommy Atkin cultivars showing significantly higher reproductive indexes relative to the 'Julie' and 'Keitt' cultivars (Table 2). The effects of pollination control treatment in 'Tommy Atkins' were significant in the indexes compared with the other cultivars (Table 2).

Table 2. Two way-ANOVA to evaluate differences in the reproduction index (IR) as a function of pollination treatment (self, cross, control) and cultivar ('Julie','Keitt', 'Kent', and 'Tommy Atkins') in *M. indica*.

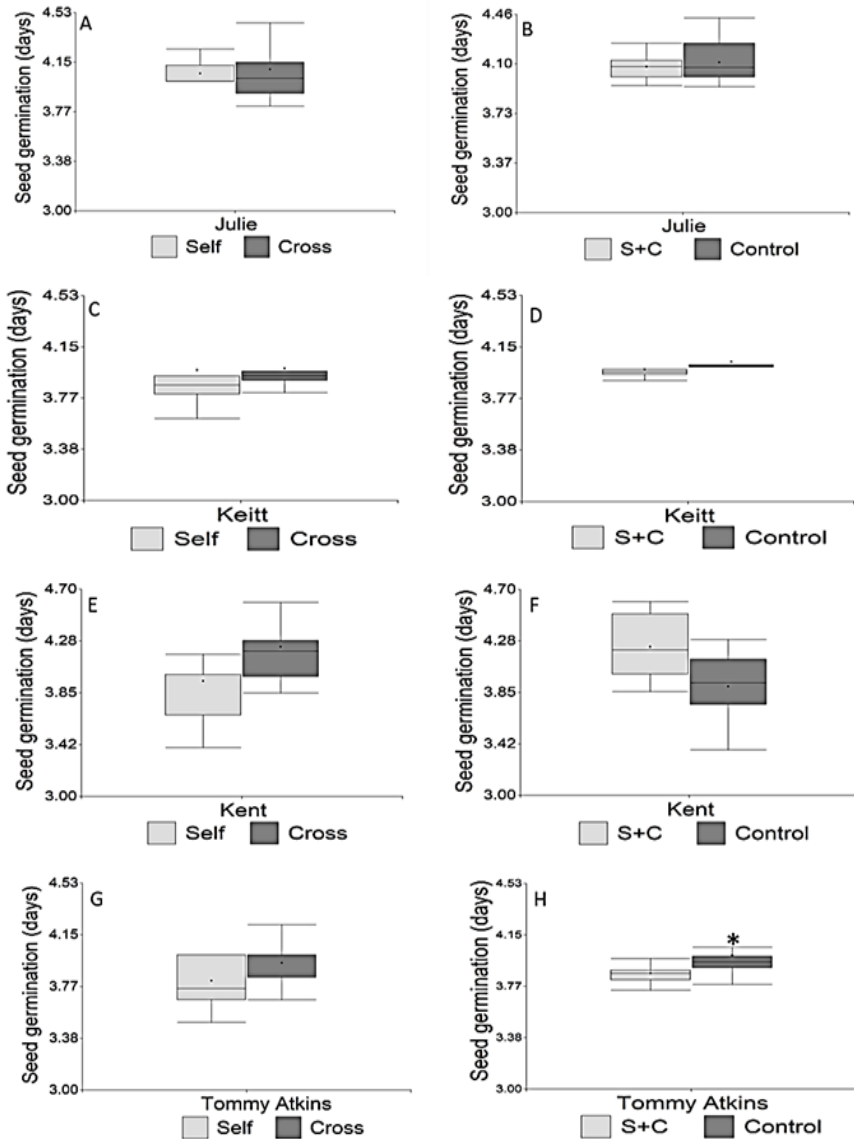| Cultivar	Mating System	Index IR Mean ± S.E.
'Julie'	self	11.37 ± 13.62 b
	cross	21.76 ± 13.62 b
	control	56.96 ± 13.62 b
'Keitt'	self	16.20 ± 13.62 b
	cross	15.75 ± 13.62 b
	control	54.56 ± 13.62 b
'Kent'	self	40.26 ± 16.28 b
	cross	54.94 ± 16.28 b
	control	81.01 ± 15.23 b
'Tommy Atkins'	self	29.11 ± 13.62 b
	cross	50.00 ± 13.62 b
	control	169.75 ± 13.62 a
Model	***F***	***p***
Cultivar	10.71	0.0001
Treatment	25.75	0.0001
Cultivar*Treatment	3.87	0.0016

Figure 6. Differences in seed germination (i.e., number of days to seed germination) between pollination pairs in four different cultivars of *M. indica* ('Julie', 'Keitt', 'Kent', and 'Tommy Atkins'). Data was analyzed with paired t-tests with the exception of the 'Keitt' cultivar which was analyzed with a Wilcoxon test. Significant differences at $\alpha = 0.05$. were indicated with "as a fecution on pollination treatment *".

Discussion

Crops breeding system is vital knowledge for successful crop management. Breeding systems in *M. indica* appear to be highly variable and understudied given the large number of cultivars available (>1000; Freeman and Sánchez 2022) and the number of cultivars studied approximately N=173 (Bally et al. 2009, Freeman and Sánchez 2022). Pollination experiments in this study set out to test whether the four mango cultivars evaluated within a germplasm maintained at the JDES, depended on animal pollinators for successful fruit production. The work also addressed the hypothesis that the mating type (self vs cross) during pollination would affect fruit production, fruit, and seed traits. This was based on the untested premise that different pollen donors may generate differences in the genetic makeup of resulting embryos that in turn could influence the likelihood of setting fruit and traits observed in fruits and seeds. In studies with other some mango varieties, the probability of fruit set in *M. indica* has been shown to increase with self-pollination (Huda et al. 2015, Gehrke-Vélez et al. 2012, Ramirez and Davenport 2016) while in others the same has been true with cross pollination (Sharma and Singh 1972, Dag et al. 1998, Dag et al. 2000). Open pollinations have been more effective than artificial pollinations in different studies (Roemer 2011, Gehrke-Velez et al. 2012, Saeed et al. 2016).

The findings of this study on the breeding system of *M. indica* indicated that all four cultivars evaluated at JDES (i.e., 'Julie', 'Keitt', 'Kent', and 'Tommy Atkins') while self-compatible are highly dependent on animal pollinators. For most traits evaluated, the mating type through controlled artificial pollinations (self vs cross-pollination within a cultivar) failed to have a significant effect on reproductive success. Yet, the effect of pollen donor cannot be discarded. Indeed, open natural pollinations tended to produce more fruit and yielded seeds with faster development times than artificial pollinations in three out of the four cultivars (i.e., 'Julie', 'Tommy Atkins' and 'Keitt'). They also resulted in faster seed germination in two cultivars ('Julie' and 'Tommy Atkins'). The combined results may suggest that not only animal-mediated pollination is needed for these cultivars but that this mode of pollination also results in improved yields, fruit, and seed traits. Below, we discuss several hypotheses that may explain these results based on other studies as well as the implications of the different results presented to the management of this mango germplasm.

One of the studies of breeding system in different cultivars of mango examined 429 open flowers of various cultivars of *M. indica* exposed to natural pollinators and found that the number of pollen grains in pistils is relatively low ($\overline{X}$: 1.2 grains/pistil, Popenoe 1917) which suggest that levels of pollen deposition are low under natural conditions (Popenoe 1917). In this study,

artificial pollination was performed by saturating stigmas with pollen to ensure pollination. Even then, open pollinations as more effective at promoting fruit production. Such a result suggests that pollen amounts do not explain differences between open and artificial pollinations "per se". That result also suggests that the observed reproductive advantage of open vs artificial pollinations may occur through various mechanisms. That may or may not be genetic in nature and that may involve other factors not considered by this study. For example, the cultivars studied here are embedded in a living germplasm collection that consists of 84 cultivars. While controlled pollinations were done with pollen from the same cultivars, open pollinations may carry pollen from other cultivars and influence the genetic composition and phenotypic expression of resulting embryos through hybridization in positive ways. Exogenous pollen could also come from *M. indica* trees available outside the germplasm, as mango is a naturalized species in the Puerto Rican landscape and one of the most common in secondary forests (Marcano 2017). Another possibility for the differences between open and artificial pollinations is that artificial pollinations may, in some cases, result in reduced fruit set if flowers are fragile or susceptible to mechanical manipulation (Sturrock 1944, 1961). Young and Leding (1954) indicate that it is not very easy to perform manual pollinations in mango. In this study, manipulated flowers did not fall right away following the experiments but had to be covered with mesh which was not the case for flowers with open pollinations. While this is a typical set up for these kinds of experiments (Eckert 2010, Huda et al. 2015) it may have created an unfavorable micro-environment for pollinated flowers. Inadequate timing of receptivity (pollinating immature stigmas with mature pollen and vice versa) may influence the fate of artificial pollinations in *M. indica* (Dag et al. 2000), we are confident that this was not a factor affecting results in our study. We ensured that our study included only mature anthers and pollen to prevent this.   In some mango cultivars (i.e., Irwin, Beni-'Keitt' and Amrapali) open pollinations have resulted in a higher probability of fruit set (Honsho et al. 2012, Srivastav et al. 2014, Amin et al. 2015). Meanwhile in a study by Saeed and collaborators (2016) in a mango cultivar without name they indicated the open pollinations led to larger fruit sizes and weights relative to the treatments with and without pollinators. Thus, one possibility is that the observed differences between open and artificial pollinations in our study are explained by large differences in pollen pools (i.e., pools more variable in open pollination and less variable in artificial pollination).

On the surface, the lack of differences between the reproductive success of self vs. cross pollination treatments in the four cultivars of mango, may appear as a paradox given that in obligate animal-pollinated systems genetic loads (i.e., high frequencies of recessive deleterious alleles) may be high (Armbruster and

Rogers 2004). Yet, the lack of differences between the reproductive success of self vs. cross pollination treatments could also be a byproduct of the process of domestication and generation of these cultivars. The loss of genetic diversity (i.e., genetic erosion) in agricultural crops is a known phenomenon that has occurred during the process of a species domestication but was exacerbated with modern agricultural practices (Smýkal et al. 2018). Artificial selection of particular crop phenotypes based on the desirability of particular traits (fruit color, size, shape, weight of fruit) leads to the propagation of individuals that will have a reduced gene pool, relative to their wild counterparts (Warschefsky and Wettberg 2019). This process not only reduces genetic variation but may also purge artificial populations of recessive deleterious alleles which in turn could explain the lack of differences between self- and cross-pollination treatments in reproductive success and in the expression of some traits in this study (fruit set, fruit development, fruit weight, seed germination, days of seed germination). It should be noted, however, that differences between self-and cross-pollination treatments do occur in many other mango varieties and that levels of self-compatibility are highly variable among cultivars (Singh et al. 1962, Davenport 2009, Popenoe 1917, Maheshwari 1934, Mallik 1957, Singh 1997, Singh and Sharma 1972). This suggests that at least for *M. indica*, genetic erosion does not occur equally across cultivars within the species and that may relate to the context of domestication and the genetic origins of different cultivars among other factors.

The original hypothesis in this study considered different reproductive responses of cultivars evaluated on the premise that artificial selection leads to genetic differentiation among cultivars. It also considered the possibility that cultivars generated in Florida (i.e., 'Keitt', 'Kent', 'Tommy Atkins') would be different from the one generated in the Caribbean ('Julie') on the premise that Floridian cultivars may have common genetic origins. Indeed, a recent genetic analyses of mango cultivars worldwide, showed that Floridian cultivars were genetically distinct (i.e., lower diversity values) and different from Caribbean cultivars (Warschefsky and Westberg 2019). The cultivars studied here showed significant differences among them in fruit production, fruit, and seed traits. However, the expected similarities and differences among cultivars based on domestication and historical origin (Floridians vs Caribbean cultivars) of the cultivars did not always hold. In addition, even when the reproduction of Floridian cultivar behaved similarly in some respects, these responses did not necessarily match those observed for the same cultivars kept in the Florida germplasm. For example, our results showed that for 'Tommy Atkins' and 'Keitt' at JDES, the mating system had no bearing on fruit set (equal success for cross- and self-pollinations) and that behavior is different from those same cultivars in Florida where self-pollinations improved the likelihood of setting

fruit over cross pollinations (Ramirez and Davenport 2016). The occurrence of somatic mutations and intra plant genetic variations in trees that make up the germplasm cannot be discarded either as a possible driver of these differences. All trees that make up the JDES germplasm originated from vegetative material (i.e., scion) that was grafted into local rootstock (i.e., original tree base planted from seed) from the Caribbean mango material present on the island (Eugenio Toro, personal communication).

Agricultural studies acknowledge the possibility of interactions between the scion (graft) and the rootstock that may influence plant characteristics (Prassad et al. 2018). Such interaction may have developed in the germplasms studied here given their age (60 years). Breeding objectives through grafting often include achieving the following: a) early generation cycle, b) dwarfing growth habit ability, c) high yielding ability, d) good fruit quality, e) resistance to biotic and abiotic stresses nature, f) tissue compatibility and, g) resistant to biotic and abiotic stresses (Prassad et al. 2018). In mango, such studies are scarce but at least one studied the relationship between different rootstock type and grafting success of the Apple mango cultivar (Beshir et al. 2019) and the relationship between rootstock on scion on yield in the Kensington Pride cultivar (Bally 2011). Both studies evaluated the effect on the scion. In the Apple cultivar, results showed that the time of grafting increase the diameters, length, and numbers of leaves of the scion, while in Kensington Pride some rootstocks increased the yield. No study has evaluated the rootstock effect in fruit traits in *M. indica* but at least in one study with the crop *Citrus reticulata* results suggest that at least for some crops there can be reciprocal interactions between the rootstock and the scion that can affect fruit traits (yield, weight, quality) three years after the grafting (Tietel et al. 2020). In that study they had to wait three years for C. *reticulata's* grafting developed and found Therefore, the potential for rootstock-scion interaction effects on fruit set in *M. indica* cannot be discarded and needs to be considered in future studies attempting to describe the role of pollen type and stock origin on crop reproductive success in perennial crop germplasms such as mango.

Another limitation of the study was the inability to explore the role of individual trees within a cultivar as pollen donors in the artificial pollination experiment given how time-consuming hand pollinations is. Also, the design was not set up to detect the pollen donors in open pollination. The effect of pollen donor on fruit traits (i.e., xenia) is an important concern of crop management (Bulant et al. 2000). In this work, pollen donor effects were seen in subtle ways when comparing artificial and open pollination (within cultivar). As stated before, the origin of pollen in the open pollinations can be diverse and include multiple parents within a cultivar and parents from multiple cultivars depending on the pollinator behaviors. From a commercial perspective, the ideal

mango fruit weights between 350 and 400 (g) and has short development time (Bally et al. 2009). Mango cultivars with seeds that have short germination times will also be preferred because of the effect of dehydration (Corbineau et al. 1986).It should be noted that for 'Keitt' and 'Tommy Atkins', cross pollinations did produce heavier fruits than the other pollination types and heavier than the ideal mango fruit weight ($\bar{\mathbf{X}}$:'Keitt'= 403 g.; 'Tommy Atkins'= 409 g), which also suggests that important levels of within cultivar genetic variation even if small is still present and may influence the desired fruit weight.

Improvements in seed germination from open pollination over other pollination treatments were mostly absent except in the Tommy cultivar. However, the observed mean differences amounted to a little over three hours which from a biological context may or may not make a difference in the subsequent establishment of mango propagules and would need to be evaluated. Ultimately, open pollination which are carried out by natural pollinators were found to be an important factor in fruit production. Therefore, the management of fruit characteristics of these four cultivars should consider relying on information on local pollinator activity.

This study highlights the importance of studying the breeding system of all living germplasm. For *M. indica*, results reiterate the importance of local pollinators and the need to evaluate the circumstances by which pollen donors may influence fruit characteristics in this crop. Detailed breeding experiments that help identify which are the best parents in terms of fruit and seed traits would help advance management strategies of *M. indica* cultivars. These experiments should be complemented with molecular studies that help clarify the origin of pollen donors from open pollination. Other possible studies to be evaluated are the potential effects of rootstock-scion interactions on the breeding system and fruit production. All these studies that can be carried out in the future would be necessary to evaluate and improve the gene banks represented in the living germplasm of *M. indica*.

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