Passiflora

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sub sole sub umbra virens

THIS MONTH'S ISSUE Creating hybrids. Maypop. Flow cytometry. Josiini moths. Growing in Italy. Sea bass recipe *and more....*

Passiflora ONLINE JOURNAL

sub sole sub umbra virens

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Cover pictures: We thank Frank Rene Stausholm for his kindness in allowing to use his images in a photo gallery and as below:

Front Cover: *Passiflora* supersect. *Distephana*
Inside Cover: *Passiflora semiciliosa*
Back cover: *Passiflora vitifolia*

We invite submissions from all *Passiflora* enthusiasts, from cartoons, garden tales, recipes and growing tips to articles about new species and hybrids and reports of wild collecting trips. Please contact the editor at myles@passionflow.co.uk. Articles in any language are welcome but will be translated and published in English only.

We reserve the right to edit or refuse articles and ask contributors to note that we may be able to offer scientific peer review depending on the topic. Please note that contributors are not paid. Letters to the editor for publication are also welcome.

Note that new species should first be submitted to the appropriate scientific botanical journals so that the validity of the name is established, after which time we may carry an article about them. If you wish to register a hybrid you should apply to the Passiflora Cultivar Registrar, Robert Rice. If your application is accepted, your hybrid will be published in the Passiflora Society International Journal & Newsletter.

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Passiflora semiciliosa

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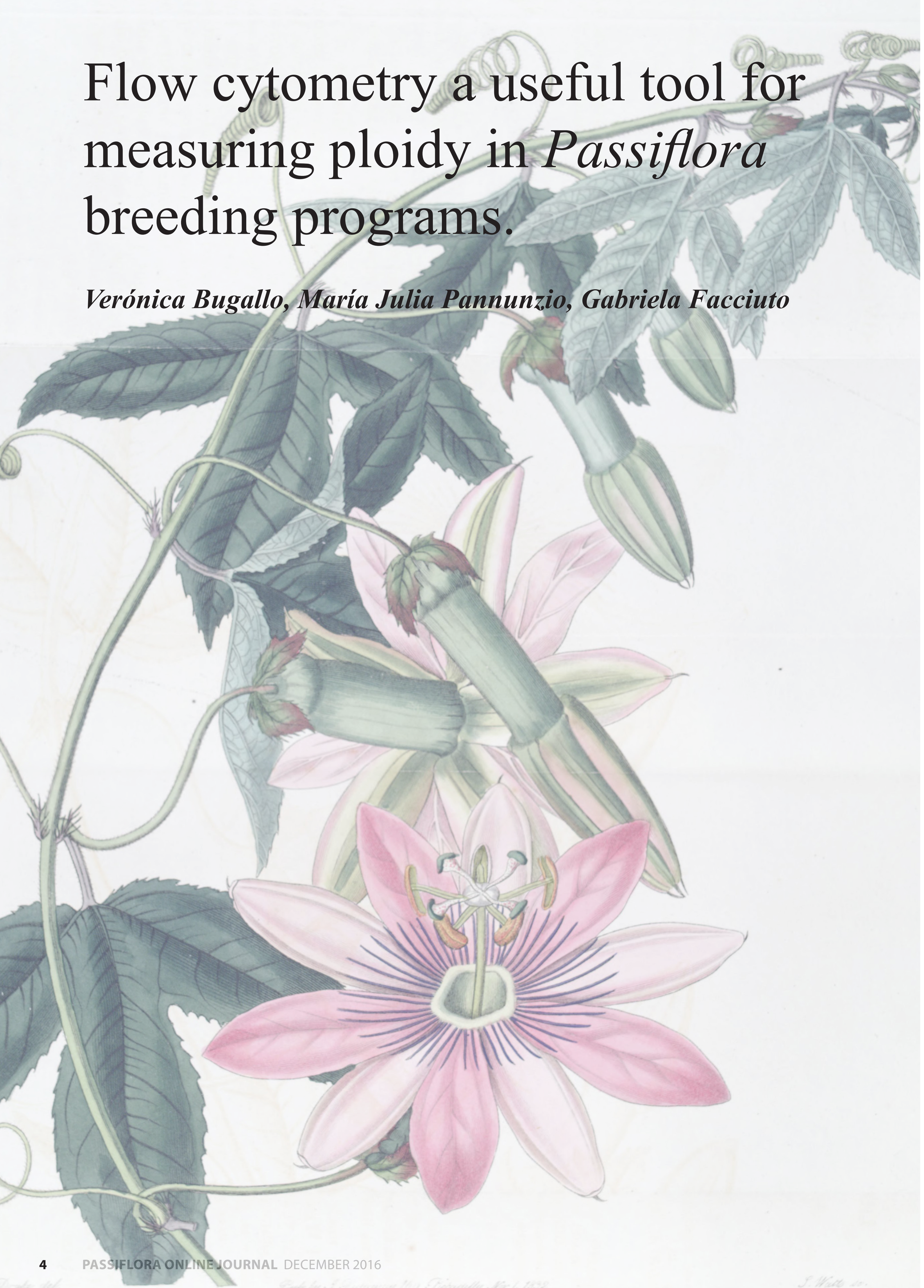

Flow cytometry a useful tool for measuring ploidy in *Passiflora* breeding programs.

Verónica Bugallo, María Julia Pannunzio, Gabriela Facciuto

Flow cytometry is used to analyse the physical and chemical characteristics of particles in a fluid as it passes through a laser. It is a fast and simple method of analysis which involves measuring the fluorescence intensity of the cellular components after they have been labeled with a fluorescent dye. On exposure to the laser they emit light of various wavelengths. This article explores the methodology and the use of flow cytometry in passionflower breeding.

Flow cytometry applied to the study of plants

Flow cytometry is a technique useful both for research with plants from natural populations that have had no human intervention and for commercial crops and plants that are part of a breeding program. The most widespread studies using flow cytometry in plants have been to analyse the genetic material that is contained in the cell nucleus.

In natural populations, this method is used to estimate the amount of DNA and its composition to evaluate genetic variability, sometimes revealing plants to be either polyploids or aneuploids. Plants are often diploid, having two pairs of each chromosome, the structures containing DNA in the nucleus of cells. In comparison polyploids may have gained one complete set of chromosomes (triploid), two sets (tetraploid) or even more. Aneuploids on the other hand have lost or gained individual chromosomes from the diploid state.

Flow cytometry was used as part of our breeding programme to confirm the induction of polyploidy by detecting the multiplication of the number of chromosomes artificially and their stability.

Sample preparation

Flow cytometry with plants requires the cell nuclei to be freely suspended in solution. Thus sample preparation involves steps to extract the nuclei of living cells.

The tissue commonly used in this type of analysis is the leaf but other tissues can also be used such as roots, pollen, stems and seeds.

The tissue is chopped (**Fig. 1a** and **1b**) and passed through special filters leaving the cells nuclei in suspension and catching the debris that could clog the fine conducting tubes of the flow cytometer (**Fig. 1.c 1.d**). With the nuclei suspension ready, samples are chosen for the various fluorescent stains (**Fig. 1.e**) to be applied. (**Fig. 1.e**) There are stains for four DNA base pairs (adenine, thymine, cytosine and guanine) which are used to determine the total amount of DNA. For example, propidium iodide is a stain used to label the nuclei in red (**Fig. 2.a** and **2.b**). Other stains include DAPI (4',6-diamidino-2-phenylindole) dye which stains adenine and thymine bases to blue (**Fig. 2.c** and **2.d.**). The stained sample of nuclei is then ready to be analyzed in the flow cytometer (**Fig. 1.f**).

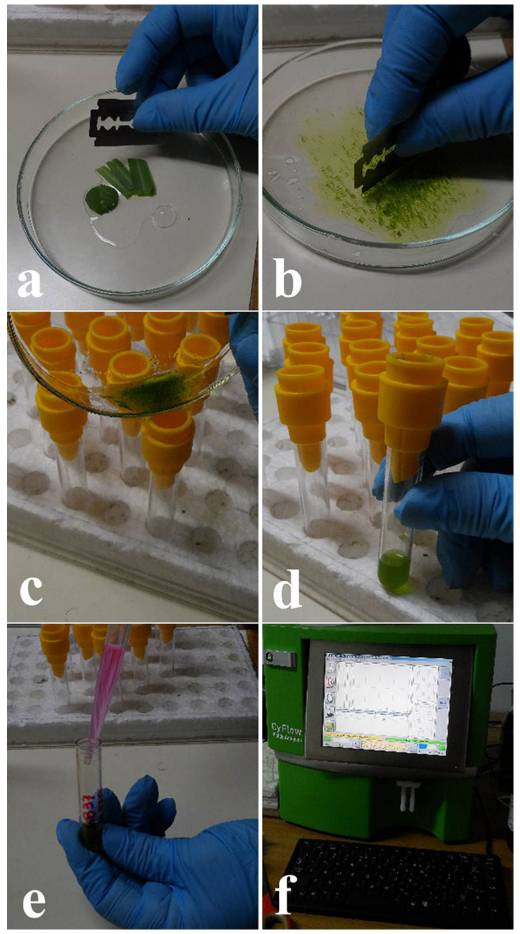

Fig. 1: Plant sample preparation for analysis by flow cytometry.
a: leaf portions of the control and plant to be analyzed; **b:** chopping up of the sample and control plants in the extraction liquid; **c** and **d:** filtration of the nuclei suspension; **e:** incorporation of propidium iodide stain (total DNA); **f:** the sample analysis in the flow cytometer.

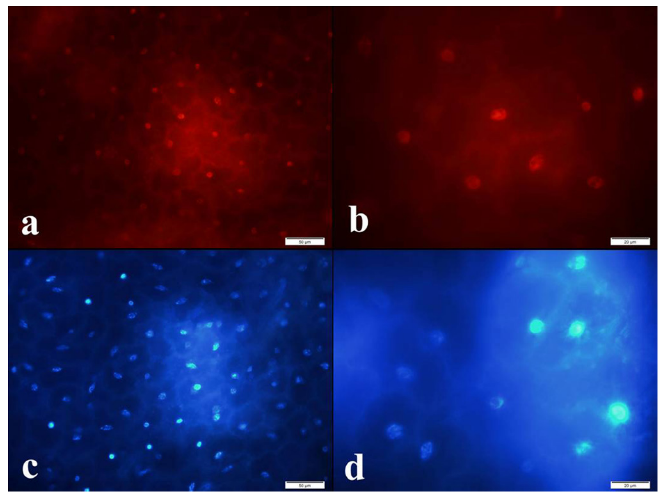

Fig. 2: Cell nuclei of the leaf of *Passiflora alata* stained with fluorescent dyes.
a and **b:** nuclei stained with propidium iodide (staining all four DNA bases); **c** and **d:** DAPI stained nuclei (stained bases adenine and thymine).

Operation of flow cytometer

Once prepared, the sample of stained nuclei is injected into the flow cytometer. This device has a series of tubes that that lead single nuclei one by one past the 'interrogation point' where each nucleus is scanned with a laser to excite the fluorescent dye in the DNA, emitting photons that are collected by an optical system. The optical signals are converted into electronic signals, converting the data for graphical analysis on the cytometer screen.

Results and interpretation

Each sample analyzed produces a histogram displayed by the flow cytometer, showing the number of nuclei that passed through the interrogation point and their fluorescence intensity. The fluorescence intensity produces a peak in the histogram with a value (**Fig. 3**). If a nucleus is twice the size of another, its fluorescence is doubled, since this value is directly proportional to the amount of stained DNA. Depending on the purpose of analysis, the fluorescent dye selected can be total, dyeing all of the four bases of DNA, or partial dyeing only two bases.

Estimation of total nuclear DNA amount

To determine the total DNA amount of a passionflower all the bases of the DNA are stained. For this purpose, as stated above, propidium iodide can be used to stain all four DNA bases. In addition, it is necessary to incorporate a control plant with a known DNA amount. Portions of the two plants are chopped at the same time (**Fig. 1a** and **1b**) and the amount of DNA in the control plant is used to estimate the amount in the other plant. For example, to estimate the amount of DNA of *Passiflora amethystina*, *Hordeum vulgare* cv. "New Golden", a barley cultivar with a known amount of DNA (10.4 picograms) was used. The resulting histogram is shown in **Figure 3.** In the sample, 4226 cores of *P. amethystina* were counted with an average fluorescence of 4875, while for *Hordeum vulgare* cv. "New Golden" 665 cores were counted with an average fluorescence of 13347. Using the fluorescence data the obtained DNA amount can be calculated, in this case *P. amethystina* has 3.79 picograms of DNA.

Confirmation of hybrid plants

In plant breeding, hybridization between closely related species is used to try to combine the best features of both parents in their offspring, if possible in the first (**F1**) generation. Flow cytometry with a partial staining can confirm the success of the cross between two plants if they have different amounts of DNA. As every male pollen donor gives the offspring plant half the genetic material from the nucleus, then the hybrid will have an intermediate amount between both parents.

For example, *P. alata* (**Fig. 4a**) and *P. caerulea* (**Fig. 4c**) were crossed to evaluate the possibility of obtaining the flower color of the first combined with low temperature tolerance of the second. Success in hybridizing can be noted by color of the hybrid flower (**Fig. 4-b**), flow cytometry can

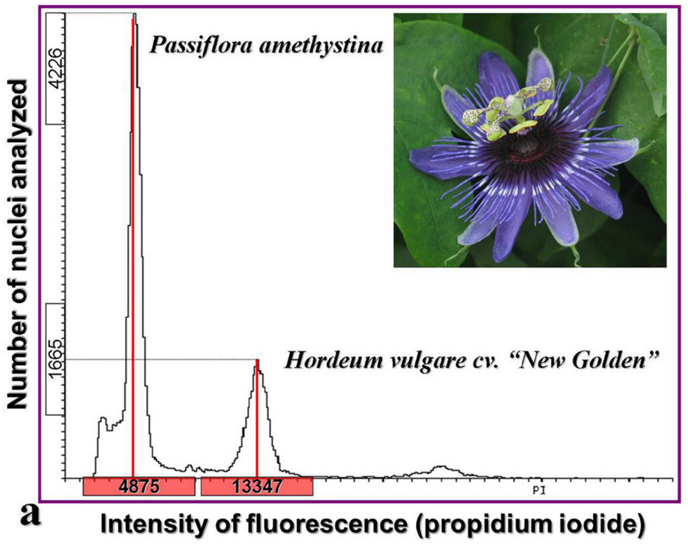

Fig. 3: Histogram for the estimation of the amount of DNA of *Passiflora amethystina* by flow cytometry (total fluorescent staining, propidium iodide).
Formula: (DNA of *P. amethystina* = *Hordeum vulgare* cv. "New Golden" DNA × *P. amethystina* fluorescence/b *Hordeum vulgare* cv. "New Golden" fluorescence)
Data: DNA *Hordeum vulgare* cv. "New Golden" = 10.4 picograms;
P. amethystina fluorescence = 4875
Hordeum vulgare cv. "New Golden" fluorescence = 13347
Result: DNA *P. amethystina* = 10.4 × 4875/13347 = 3.79 picograms

confirm that there has been a successful cross long before the blooms (**Fig. 4d**).

Detection of induced polyploidy

The induction of polyploidy by chemical means, is used in breeding programs as doubling the amount of DNA of a plant will increase the size of its cells. This may lead to stronger rapid growing thicker root systems, vigorous plants, striking sturdier foliage, bigger flowers with wider petals and sepals, flowers that stay open longer and superior hardiness. Treatment is not always successful and it is desirable to have a method to confirm whether a plant is polyploid or the attempt failed.

Flow cytometry can detect polyploidy because a plant with twice the amount of DNA per nuclei will have twice fluorescence. For example, in the hybrid (*P.* 'Amethyst´ x *P. caerulea*) x *P. amethystina* a treatment was applied to obtain larger flowers (**Fig. 5.a**). The results were evaluated by flow cytometry. Histograms of untreated plants and treated unsuccessfully showed fluorescence value 50 (**Fig. 5.b** and **5.c**) while this value for induced polyploids was 100, twice the previous (**Fig. 5 d**).

Future of flow cytometry in plants

While flow cytometry has been a useful tool in the

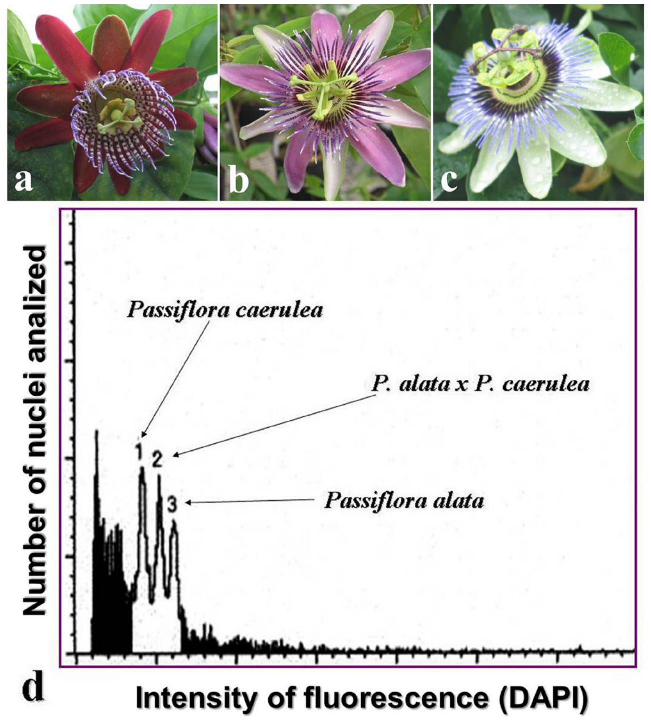

Fig. 4: Detection by flow cytometry of a hybrid of *Passiflora alata* and *Passiflora caerulea* (DAPI fluorescent staining partial).
a: *P. alata*; **b:** interspecific hybrid *P. alata* x *P. caerulea*; **c:** *P. caerulea*; **d:** histogram of the number of nuclei analyzed according to their DAPI intensity of fluorescence for a combined sample of *P. alata* (♀) + *P. caerulea* (♂) + *P. alata* x *P. caerulea* (hybrid).

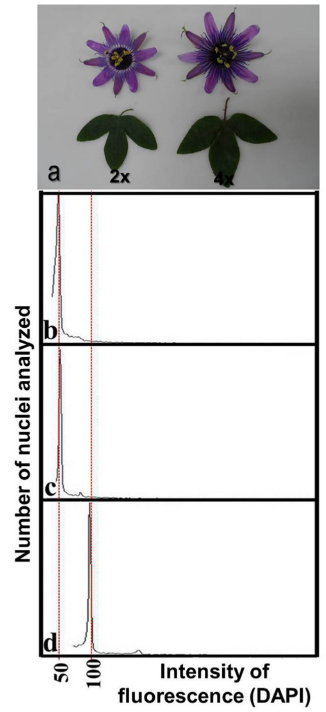

Fig. 5: Detection of induced polyploids by flow cytometry.
a: Flower and leaf of the diploid hybrid (*P.* 'Amethyst´ x *P. caerulea*) x *P. amethystina* (2x) and its induced polyploid (4x); **b, c** and **d**: flow cytometry histograms for untreated plants (2x); unsuccessfully treated plant (2x) and obtained polyploid plant (4x), respectively.

determining of the ploidy of plants in the breeding process, there are still unstudied applications. Future research, in both genetics and plant physiology, will surprise us with new uses for this technique.

Literature on the subject

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Passiflora incarnata L., 1753, 'Maypop'.

By Padric Stephenson

My fascination with Maypops, probably the most variable and confusing of all the passionflowers, began when I was 7 years old. My first experience was finding one in bloom in the hedge row of our home and being spellbound by its beautiful fragrant flower. Then later, as we kids were playing army in the 1950s, we used the fruits as grenades. If you got hit by one and it popped, you were dead. Fast forward to my adult years, I would see them on the side of the road and sometimes stop to admire them. In 2006 I started adding passionflowers to my plant addiction and got really interested in hybridising them. I was particularly attracted to the tropical red passionflowers and so wanted to be able to grow one in my yard. I'm not in a zone where they would survive over winter and decided that knowing how hardy *P. incarnata* was, I could use it to add hardiness to my breeding. That set me to collecting clones of *P. incarnata* from the wild and from all over its wide range in the USA.

A little info on *P. incarnata* per Wikipedia:

"*Passiflora incarnata*, commonly known as Maypop, purple passionflower, true passionflower, wild apricot, and wild passion vine, is a fast-growing perennial vine with climbing or trailing stems. A member of the passionflower genus *Passiflora*, the maypop has large, intricate flowers with prominent styles and stamens.

One of the hardiest species of passionflower, it is a common wildflower in the southern United States. The Cherokee in the Tennessee area called it ocoee; the Ocoee River and valley are named after this plant, which is the Tennessee state wildflower. This, and other passionflowers are the exclusive larval host plants for the gulf fritillary and non-exclusive for the variegated fritillary butterflies.

The stems can be smooth or pubescent; they are long and trailing, possessing many tendrils. The leaves are alternate and palmately 3-lobed and occasionally 5-lobed, measuring 6-15 centimeters (2.4-5.9 in). They have two characteristic glands at the base of the blade on the petiole. Flowers have five bluish-white petals. They exhibit a white and purple corona, a structure of fine appendages between the petals and stamens. The large flower is typically arranged in a ring above the petals and sepals. They are pollinated by insects such as bumblebees and carpenter bees, and are self-sterile. The flower normally blooms in July.

The fleshy fruit, also referred to as a maypop, is an oval yellowish berry about the size of a hen egg; it is green at first, but then becomes orange as it matures. As with other *Passiflora*, it is the larval food of a number of butterfly species, including the zebra longwing (*Heliconius charithonia*) and Gulf fritillary (*Agraulis vanillae*). In many cases its fruit is very popular with wildlife. The egg-shaped green fruits 'may pop' when stepped on. This phenomenon gives the *P. incarnata* its common name. The maypop occurs in thickets, disturbed areas, near riverbanks, and near unmowed pastures, roadsides, and railroads. It thrives in areas with lots of available sunlight. It is not found in shady areas beneath a forest canopy."

I have collected *P. incarnata* all over the eastern lower quarter of the state of Alabama and have bought them from most of its range as well as having had many gifted to me. One thing I can say is, no two are identical in flower. Even in a large area of a population there can be many variations in flower color and size as well as form. I have also noticed that its growing conditions may have an effect on the color saturation and flower size. About a third of my twenty or so collected plants, after a year in pots with good conditions and fertilizer, bloomed with completely different colored and sized flowers. The images show many of the variations that I have found that have proved to be stable after potting and growing in my conditions, as well as a composite picture of many clones collected all over North Georgia.

Passiflora breeder Ethan Nielsen has discovered polyploid species of *P. incarnata* in the wild. He has collected in Florida some that are tetraploid and a few that are triploid. I haven't found any polypoid ones here in Alabama, but think it was because I didn't know how to recognize them out of bloom. For a long time there were only a couple of white (apparently not pure albas) *P. incarnata* in the trade for breeders to work with, now because of Ethan Neilson and a couple of other *Passiflora* growers I now have a collection of eight. One of which is a tetraploid and am using it in my breeding. One thing I notice is *P. incarnata* seems to be very dominant in its habit of sprouting from root sprouts and transfers that to its hybrids.

I have found wild *P. incarnata* growing in everything from sand, to clay, to rich soil fields and it seems to only require good drainage to grow well. I have grown them in pots for 5 years and as winter approaches and they go dormant, I move them into the greenhouse. They will remain dormant all winter, only sprouting back up as soon as the sun warms the greenhouse to above 80°F (26.6°C) during the day. I have also left them sitting outside exposed to the elements and had them come back in the pots, but they were later in coming up than those in the greenhouse.

They don't seem to respond to a lot of fertilizer with more growth and blooms. They do however respond early in the season to a good balanced time release fertilizer and really don't need any more supplementation. I do know of a few *P. incarnata* growing beyond their recognised hardiness zones in the USA. The map (p. 20) shows the normal range for them, but there are populations that have adapted to much colder areas. They have also been spread to the west coast of America by *Passiflora* enthusiasts. I recommend using them for cold hardiness if you live in an area that gets hot summers. They really don't seem to grow well in England as they tend to have much cooler summers and the winters are too wet. I hope maybe you can gather some useful information from this brief article.

Passiflora incarnata photo gallery

By Padric Stephenson

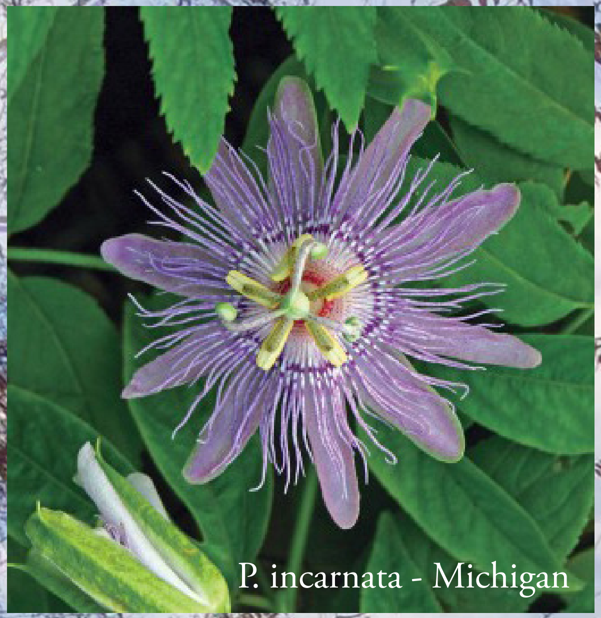
P. incarnata - Florida

P. incarnata -
Missouri

P. incarnata - Central Tennessee

P. incarnata - Florida

Wild collected tetraploid
P. incarnata - Florida

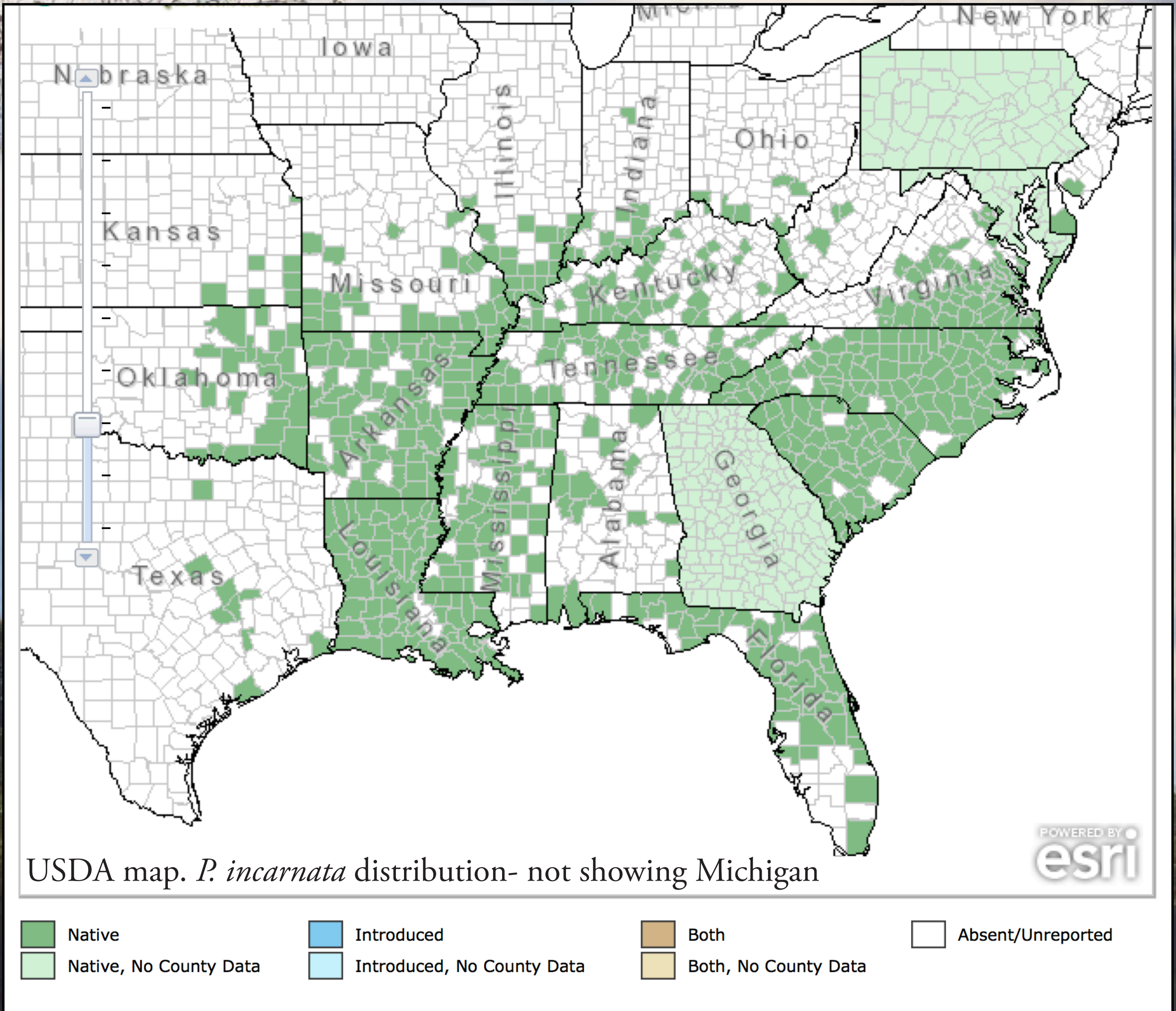


P. incarnata - Michigan

P. incarnata - Florida

P. incarnata - Alabama © T. G. Barnes

P. incarnata **-** Ohio



USDA map. *P. incarnata* distribution- not showing Michigan

P. incarnata variations - North Georgia
© Nick Batora

Passiflora incarnata - Texas

By Hal Love

Leaf showing petiole gland position indicative of *P. incarnata* species

P. incarnata foliage and fruit

P. incarnata flower variations

P. incarnata - cut ripe fruit

P. incarnata - fruit ripening

Josiini moths (Lepidoptera: Notodontidae: Dioptinae), an overlooked 'passiflorivorous' guild of Notodontidae moths from the American Tropics

By Andrés M. Orellana B. aorell@gmail.com
Universidad Nacional Experimental de Táchira

Passion vines exhibit their greatest diversity in the tropics of the New World (Vanderplank 2000). In this region *Passifloraceae* display variety not only in species, but leaf shapes, flowers and fruits color, disposition of extrafloral glands, tendrils, and even their general architectural morphology, as evidenced by the inclusion in this group of the *Astrophea*, modest to large sized trees with a respectable diameter at chest height. Passion vines are even diverse in flavor of their fruit.

An intense interaction exists between herbivores and their host plants where both are struggling for their existence. Such interactions are an important basis for defining a guild or array of herbivorous species that exploit a particualr resource. These interactions have been echoed by an axiomatic discipline in evolutionary theory, namely coevolution, a mutual struggle among interacting partners, that can aptly be described as an "arms race". Escaping from herbivore harassment leads to innovations in the defensive system of the plant. In turn that encourages the herbivore to innovate and adapt in order to survive. This spirals into an ever increasing and complex struggle (Ehrlich & Raven 1964). This force synergistically drives the evolution of organisms and helps explain the appearance and interactions of new species in time.

Perhaps the best documented coevolutionary interaction between plants and herbivores is between the Passion vine butterflies from the subfamily Heliconiinae and plants from the family *Passifloraceae* (Ehrlich & Raven 1964, Brown Jr 1981, Ackery 1988). It is known, with very few exceptions, that Heliconiine butterflies (also known as longwings) are strict feeders on plants of *Passifloraceae*, and hence the term "passiflorivorous" for this guild.

Passion vines are an important resources for these butterflies not only because they serve as food for their caterpillars, but because they in turn use the plant's phytochemistry to provide the building blocks for a form of chemical warfare against their predators. They do this by changing their physiology, either by chemical sequestration or de novo chemical synthesis.

Other than Heliconius butterflies, a relatively larger cluster of Lepidoptera also rely on *Passiflora* to survive. Neotropical day flying moths in the tribe Josiini of the Notodontid subfamily Dioptinae (containing 456 described species in 32 genera), form a monophyletic clade of species that largely feeds on *Passifloraceae* plants (Miller 1991, Miller & Otero 1994, Miller 2009). So far *Passifloraceae* herbivory has been recorded for at least 31 Josiini species within seven genera (containing 125 species in 11 genera in all). This includes *Passifloraceae* species in subgenera *Decaloba*, *Astrophea*, *Tacsonia*, *Granadilla*, *Granadillastrum*, and *Distephana*, the greatest bulk of species preferring *Decaloba* (Miller 2009), however, there is still much to be learnt about the entire scope of the herbivory of passion vine moths.

It is known that Dioptinae derive from nocturnal ancestors and are positioned in an advance state or derivation within Notodontidae (Miller 1991). Dioptini, the second and extant tribe within Dioptinae, raise their caterpillars in a wide array of unrelated foodplants. Such resources however, are similar to those commonly recorded for butterflies in general, ranging from Melastomataceae, Fagaceae, Sapindaceae, Poaceae, Malvaceae, Arecaceae and many more (see Miller 1992 and Miller 2009). It is clear that Josiini evolved using the order Malpighiales as foodplants (which includes *Passifloraceae*).

Josiini are diurnal and their colourful wing patterns (**Figures 1 and 4)** act as a warning signal to predators that they are unpalatable, possibly as a result of sequestration of the cyanogenic glycosides that they they take in from the passion vines. Chemistry as a defense against insects generally acts by causing unpleasant sensations that predators will associate with a ubiquitous signal, in this case color. Despite the fact that tests are needed to analyse the obnoxious characteristics of Josiini as food items for vertebrate predators, it has been proven from experiments (Orellana 2000) that Giant golden web spiders (*Nephila clavipes*) systematically reject Josiini and expels tangled moths unharmed from their orb webs. This may be as a result of both chemical and visual cues, as it has been shown that Jumping spiders (Salticidae) discriminate prey by vision (Jackson & Pollard 1996). Despite being smaller insects than the Heliconiinae, and hence eating less plant material per individual, Josiini caterpillars may attack and defoliate passion vines during sudden population outbreaks. (**Figures 2 and 3).**

Figure 1. Plate 71 of volume 6 from Hering (1925) in Seitz: *Macrolepidoptera of the World*, depicting colorful Josiines and most common wing patterns.

Severe defoliation is a rather common behavior in Notodontidae, and particularly by some Dioptinae. For instance, the Californian oakworm, *Phryganidia californica* (Dioptini) regularly produces severe infestations during outbreaks and may become serious forest pests (Harville 1955). This also has been documented for tropical species, such as *Scotura annulata* (Wolda & Foster 1978). On a short term visit to Panamá I had the opportunity to testify to the latter event, and moreover, following a relatively long dry spell in 1998, another two Dioptinae species were also observed to coincide in a noticeable outbreak, *Scotura nervosa* on *Hybanthus prunifolius* (Violaceae) and *Iphialtias draconis* feeding on *Turnera panamensis* (Turneraceae). The first of these moths is identifiable as a Dioptini and the latter as Josiini.

In Venezuela, I have seen similar outbreaks of *Josia megaera*, baring foliage and stems of an aromatic *Turnera* species. Natural populations of several *Josia* will defoliate their host plants severely, such as *J. radians* on *Passiflora manicata* and *P. capsularis*, as well as *J. insincera* on *P. biflora*. Native species will also attack and cause similar damages on cultivated exotics. Venezuelan *J. radians* attacks mesoamerican *P. anfracta* and *P. helleri* (M. Molinari com. pers.) denuding leaf blades down to their primary veins.
Bearing the above in mind, the high Andean Josiini *Scea necyria*, which causes major defoliation in its natural habitat, was put forward in a failed attempt to control *Passiflora tarminiana (*previously misidentified *as P. tripartita var. mollissima)* in Hawaii, where it is an invasive aggressive weed, (Campbell et al. 1993). (**Figure 4c).**

Josiine moths have also been recorded as feeding on the related Violaceae and Achariaceae, reflecting that the group has successfully colonized a clade of plants (order Malpighiales). Whether or not the moths use these hostplants in a similar fashion as the longwing butterflies is a matter that requires more research, and overall, the field offers a vast amount of challenging natural history that has yet to be investigated.

My thanks goes to Miguel Molinari for reading and commenting on this article. To Jesús Linares, Ivo Kindel, Sergio Monteiro, André Maurino, John Smiley and an anonymous photographer at the McGuire Center for Lepidoptera and Biodiversity for their kind use of their images.

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Figure 2. *Josia gigantea* on *P. costaricensis* © John Smiley

Figure 3. Full grown final (4th) instar larvae of several Josiini. **3a:** *Josia insincera* on *Passiflora biflora* from San Cristóbal, Venezuela **3b:** *Lyces flavissima* feeding on *P. laurifolia* from San Cristóbal, Venezuela. **3c:** Unidentified *Scea* species feeding on an unidentified *Passiflora* from Curitiba, Paraná (Photo by Sergio Monteiro). **3d:** *Josia turgida* feeding on cultivated *P. rubra* at San Cristóbal, Venezuela (All photos by the author, except where indicated).

Figure 4. Live adult Josiini. **4a:** A Turneraceous feeder, *Josia megaera*, extending its proboscis. Montalbán, Carabobo, Venezuela (Photo: Jesús Linares). **4b:** *Lyces aurimmutua* showing the most extended color patter within Josiini and typical resting posture. Santa Catarina, Brazil (Photo: Ivo Kindel). **4c:** A mid to high Andean species, *Scea necyria*, which was tested as failed biological agent to control aggressive *Passiflora tarminiana* in Hawaii. Near Loja, Ecuador (Photo: archives of the McGuire Center for Lepidoptera and Biodiversity. Gainesville, Florida). **4d:** *Scea auriflamma* feeding on unidentified flowers. Imbituba, Santa Catarina, Brazil (Photo: Maurino André)

Passiflora species photo gallery

By Frank Rene Stausholm

Some reds and pinks

Passiflora supersect. *distephana*

Passiflora cinnabarina

Passiflora perfoliata

Passiflora semiciliosa

Passiflora murucuja

Passiflora racemosa

Passiflora glandulosa complex (flower only)

Passiflora edmundoi

Passiflora sublanceolata

Passiflora tulae

Passiflora loefgrenii 'Corupa'

Passiflora tarapotina

Passiflora vitifolia

Passiflora reflexiflora

Passiflora gritensis

Passiflora racemosa 'Buzios'

Passiflora supersect. *Tacsonia* - Ecuador

Growing *Passiflora* in North Italy (UDSA Zone 8)

By Stefania Mattiuzzo

P. 'Araba Fenice,' *P.* 'Byron Beauty' x *P.* 'Maia'

The world of *Passiflora* is one of fascinating colours and attractive shapes. In this article I am going to discuss the hardier species and varieties that can withstand relatively harsh climates.

With great regret, I often talk to people who have long since abandoned the idea of cultivating them either in a garden or on a balcony just because of one or two failures with this genre.

There are nearly 600 known species of *Passiflora*, being found in North America, Central America, South America, Asia and Australia. Accordingly, some live in colder climates than others and for hybridizing purposes it is best that we start with these species.

In my experience of growing *Passiflora*, I have found three of the hardiest to be *P. caerulea, P. tucumanensis*, *P. incarnata* and their hybrids.

All three species have the following in common:

• They produce strong pollen.

• They become herbaceous in severe winters with the stems above ground sometimes dying back. The plants will then remain dormant until the warmer temperatures of spring promote fresh growth.

• They can produce suckers which can sprout a metre or more away from the parent plant. These can be gently dug out with as much root attached as possible and potted up.

It is also possible to make cuttings from the roots themselves. Dig up a large piece of healthy root making a clean break with the main body of the plant. Using a mix of soil and sand bury the root in a pot, place it in the sun and after a few weeks a new shoot should appear.

Passiflora are susceptible to overwatering. Rather than have them on a regime of daily watering, they should only be watered when they are near dry. If planted in pots, they are best watered from underneath to prevent root rot. In winter do not water them at all especially if there is risk of frost. *Passiflora* can often survive a harsh but dry frost but wet roots are lethal. So even if the plant appears to be withering and the leaves dying back do not water.

In spring when all frost risk has past the plants can be pruned hard to remove dead wood and yellowed stems and they should come up more vigorously than the year before. At this time, although not essential, a nitrogen rich

P. 'Constance Eliott' Sweet fruit selection fertilizer will help growth. Later in the growing season fertilisers rich in potassium and phosphorus will help promote flowering.

I have chosen the following species for their hardiness.

Passiflora caerulea
The most common *Passiflora* in Italy, and certainly the most well known. In some regions it is evergreen, in others herbaceous. Native to Argentina, Brazil and Paraguay, blooming with 6-8 cm wide flowers, it is able to withstand temperatures of -15 °C. For many of us it was the first love, with its white petals and sepals, and crown with corona filaments banded dark at the centre then white and blue at the tips. Its leaves are 5 lobed and dark green. Fruits are decorative, large and egg shaped, ripening from green to yellow orange.

There is a white variety of *P. caerulea* called *P.* 'Constance Eliott'. In recent years a new selection of it *P.* 'Avalanche' has been released by Cor Laurens with similar traits but with larger flowers. I am proud to have also had the good fortune myself to select an exceptional white variety from fruit. It has grown into a massive plant and unusually for this species the fruit are particularly sweet and pleasant to eat freshly picked.

Among the best known hybrids from *P. caerulea* is the very old *P.* x *violacea*, a hybrid with the colorful and spectacular *P. racemosa*. Another very interesting hybrid is *P.* x *colvillii*, *P. caerulea* with *P. incarnata*. This combination produces interesting features without losing resistance to cold.

I would like to mention *P.* 'Drops of Heaven', my first hybrid of a few years ago, where the *P.* x *violacea* has transferred the grace of its elegant petals, while the delicately colored filaments with black and white stripes terminating with a splash of blue on the tips, give lightness and liveliness to the flower.

P. tucumanensis
Formerly known as *Passiflora naviculata*, it is a native of Argentina, Bolivia and Paraguay. It has 3 to 5.5 cm. diameter flowers, and is resistant to temperatures up to -10 °C. In most cases, it is herbaceous, tending to die back to the ground completely in the winter. This species has an attractive crown of corona filaments with alternating white and blue bands. This feature is often inherited in its hybrids, as well as the hardiness and ease of cultivation. There's also a white selection available. Hybrids of it that I enjoy the most in my personal collection include *P.* 'Jutta', *P.* 'Blue Desire' and *P.* 'Guglielmo Betto'.

P. 'Claraluna' x *P. caerulea* 'Constance Eliott'

P. 'Gocce di Cielo,' *P. x violacea* 'Victoria' x *P. caerulea*

Passiflora incarnata,
Native to the United States, its flowers are 6 to 9 cm in diameter and it can withstand temperatures up to -15 °C. There are many variations within this species, including a very beautiful white variety. The plant dies back in the winter completely losing its leaves. It is much appreciated for its flowers with long, fluffy and soft filaments, and for its resistance to cold. The beautiful hybrids of this species are some of my favourites and I love the reward of seeing a new one open for the first time. All *P. incarnata* hybrids are easily cultivated, like free draining soils, full sun and if possible, plenty of space for growth. Like *P. caerulea* they can become large specimens, particularly when grown in the ground and trained along fences. Among my favourite *P. incarnata* hybrids are *P.* 'Temptation', *P.* 'Inspiration, and some of my own hybrids of this past year, *P.*' Essence', *P.* 'Araba Fenice ', *P.*' Energie '. I have managed to cross *P. incarnata* with a number of other species in recent years and have started to reap the rewards. Some of the most recent are not yet named, such as *P.* 'Inspiration' x *P.* 'Temptation'.

I would add that as well as the other varieties that tolerate a few degrees below zero for several days, I have created a curious hybrid that I named *P.* 'Ederina', for its resemblance to our Italian Common ivy (*Ivy helix*). The parents are *P.* suberosa and *P. sexocellata*. Crossed with each other, after having grown for years in a cool greenhouse in my Italian garden in Rome, they have developed a resistance to cold that I never expected. Its flower is a delicate gem, small but still larger than *P. suberosa*, and its leaves are dark green with a slight variegation. It grows vigorously, yet it can also be contained for those with little space, and its foliage with delicate little flowers gives a beautiful covering to a wall or fence.

P. x violacea 'Victoria' x *P. caerulea* © Giorgia & Vincenzo

P. 'Energie' (*P.* Temptation x *P.* Temptation)x *P.* 'Vivacemente'

P. 'Amethyst' x *P. caerulea*

New hybrid, *P.* 'Inspiration' x *P.* 'Temptation'

P. 'Ederina'

P. 'Ederina'

Creating *Passiflora* hybrids: a process of attrition

Leslie A. King (International *Passiflora* Cultivar Registrar, 2004 – 2011) *les@king.myzen.co.uk*

Summary
This article is not about describing successful outcomes, but rather documents the many opportunities for failure that can occur in breeding *Passiflora*.

Records were kept of 271 crossing attempts made between 1997 and 2015 using 28 species and 43 hybrids. Of the 271 crosses, 76 (28%) formed mature fruits, of which 42 (15%) contained seeds. Of these, germination occurred in 23 cases (8%), of which 15 (5%) produced flowering plants.

Introduction

I have been growing *Passiflora* for about twenty years, and it was clear that these plants had an interesting evolutionary history. The creation of *Passiflora* hybrids offered a means of exploring some of that history. Furthermore, hybridisation of *Passiflora* can be a worthwhile exercise for the amateur grower. Thus: the field is not dominated by large commercial companies; the number of known species and hybrids is still reasonably modest; and hybrids will often flower within 12 months of the original cross-pollination.

One of the first passion flowers that I grew was *P.* 'Amethyst', but at the time its parentage was unknown. This sparked an investigation that eventually led via a second puzzling hybrid *P.* ×kewensis (now known as *P.* 'Kew Gardens') - to a much clearer understanding of the origin of both hybrids [1-5]. Later, Myles Irvine and I studied the genetics of polyploids. We were able to confirm the tetraploid status of a number of existing *Passiflora* taxa. More importantly, we showed that when tetraploid plants were crossed with diploids, the resulting plants were triploid, some of which were fertile [6]. Recently, an examination of *P.* 'Buzios', again based partly on its ability to form certain hybrids, led to the conclusion that *P.* 'Buzios' could be a natural hybrid [7].

At my location in South England, only *P. actinia*, *P. caerulea* and a few hybrids of *P. caerulea* are sufficiently hardy to survive most winters outdoors. Much of my collection lives in a heated conservatory. Because they have to share space with an orchid collection and other tropical plants, at any one time I can only grow around ten different passion flowers, although this small collection changes from year to year. Apart from the investigations described above and simple curiosity to see 'what works', some effort has been put into trying to create hardy plants, the best example of which so far is *P.* 'Scorpius' [*P.* 'Lunametista' (♀) × *P. caerulea* (♂)] [8].

These endeavours have confirmed the well-known observation that *P. caerulea* stands out as a species with the most useful source of 'hardy genes'. By contrast, the other two 'hardy species', *P. incarnata* and *P. actinia*, have much less to offer. The former is often claimed to be hardy, but *P. incarnata* fails to survive wet winters and persistent frosts; apart from *P. ×colvillii* [*P. incarnata* (♀) × *P. caerulea* (♂)], few of its named hybrids can be described as hardy. While *P. actinia* is almost as hardy as *P. caerulea*, it has proven difficult to hybridise successfully. Thus *P. actinia* has been recorded as producing hybrids with only five other species: *P. caerulea* (e.g. *P.* 'Anemona'), *P. incarnata* (e.g. *P.* 'Medallion'), *P. alata* (e.g. *P.* 'Olga') and *P. phoenicea* (e.g. *P.* 'Floral Fountain'), all of which were created before 2001 [9], and the recently registered hybrids with *P. kermesina* [10]. In 2008, I grew the hybrid *P. actinia* (♀) × *P.* 'Mini Lamb' (♂) from seeds produced by Myles Irvine, but it failed to flower. In 2014, I made the hybrid *P. ×violacea* (♀) × *P. actinia* (♂), which also has not flowered. There are two particular problems with *P. actinia*. Firstly it is often reluctant to flower and, secondly, it flowers outdoors in late Spring usually before most other *Passiflora*. Furthermore, it is unclear if *P. actinia* could ever produce hardy hybrids. This is illustrated by *P.* 'Anemona' [*P. actinia* (♀) × *P. caerulea* 'Constance Eliott' (♂)] which is claimed not to tolerate temperatures below 0ºC, even though its parents are both much more robust [11]. It is unlikely that the other known hybrids of *P. actinia* are hardy.

This article is not intended to provide a detailed account of successful crosses, but rather to demonstrate and quantify the many opportunities for failure.

Methods
Records were kept of 271 crossing attempts and their outcomes made between 1997 and 2015 using 28 species and 43 hybrids. The total number of species and hybrids requires some qualification:

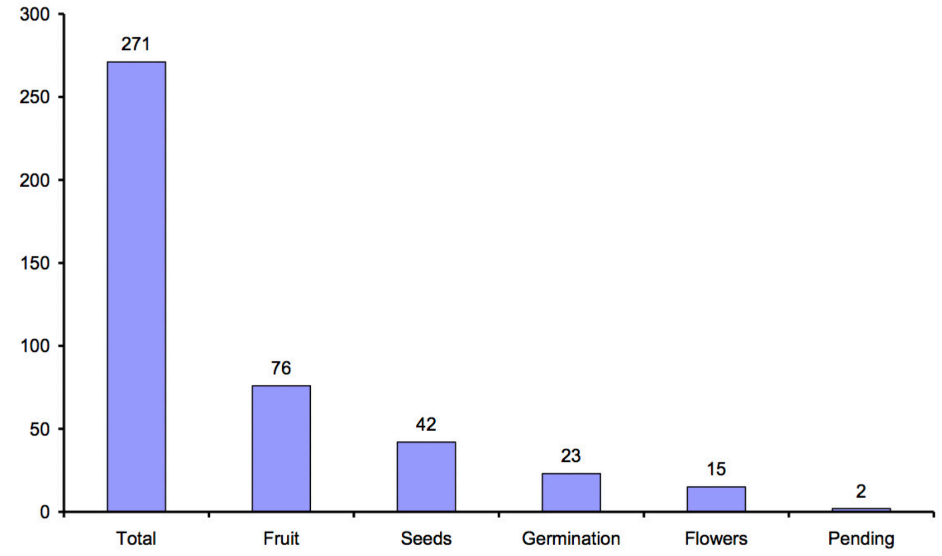
• It included two forms of *P. ×belotii*, two forms of *P. racemosa* and several clones of *P. caerulea*.

• It did not include 'embedded' species in hybrids where I have not grown that species in isolation.

• The two forms of *P. edulis* (i.e. *P. edulis* f. *edulis* and *P. edulis* f. *flavicarpa*) were treated as separate species.

• Diploid and tetraploid forms of *P. tucumanensis* and *P. caerulea* were counted as separate species.

Figure 1 Attrition in creating *Passiflora* hybrids showing the fate of the original 271 crossings. The category 'Pending' shows crosses from 2014 that had germinated but not flowered by mid-2016.



• When different clones of a species were used in hybridisation experiments they were regarded as separate crosses.

• *P.* 'Buzios' and *P.* 'Carioca' (a selection of *P.* 'Buzios') were treated as hybrids and not forms of *P. racemosa* [7].

Of the 28 species and 43 hybrids used, 61 (86%) had a parentage in sub-genus *Passiflora*; the remaining ten were from sub-genus *Decaloba*. Of the 61 taxa from sub-genus *Passiflora*, 6 were tetraploids, one was a triploid and 54 were diploids of which 4 had a parentage in Supersection *Tacsonia*.

A number of otherwise successful cultivars were excluded as follows:

• Uncontrolled (bee-pollinated) crosses and those where the pollen parent was uncertain;

• Plants grown from hybrid seed produced by others since there was no means of knowing how many failures preceded the production of such seed;

• Siblings of previously created hybrids;
• Intraspecific crosses (e.g. *P. caerulea* Taxon #1 × *P. caerulea* Taxon #2)

• Two instances of apomixis. When pollinated by *P. caerulea* or *P. racemosa*, *P. loefgrenii* 'Corupa' yielded plants that were identical to the female parent.

Although the above list excluded a number of otherwise worthwhile cultivars, this level of selection was necessary to avoid distorting the statistical analysis described here, which was based strictly on the success or failure of crosses. Wherever possible, at least 4 flowers were pollinated in each hybridisation attempt. Of the many thousands of crosses that were theoretically possible using these 71 taxa, only a small fraction could be achieved. Thus many hybrids had no visible pollen, and not all plants flowered at the same time or were even grown in the same year. Most were original crosses, but a few were attempts to create new examples of existing hybrids. Most pollinations were carried out under glass where bees and other insects could be excluded. When pollination of outdoor plants was necessary, precautions were taken to avoid contamination by the ubiquitous pollen from *P. caerulea*.

Results
The hybridisation process was divided into four stages:

1. Fruit formation. Of the 271 crosses, 76 (28%) formed mature fruits. In all other cases, either no fruit formed or immature fruit aborted.

2. Seed formation. Of the 76 crossings that produced fruits, 42 contained seeds; the remainder were empty or contained immature seeds.

3. Germination. Of the 42 crosses that produced seeds (15% of the original 271), germination occurred in 23 cases (8% of the original 271).

Table 1 Classification of the 271 hybridisation attempts and their outcome

Crossing type	Total	Fruit	Seeds	Germination	Flowers	Pending
Species (a) (♀) × species (b) (♂)	36 (13%)	13	11	5	4	0
Hybrid (♀) × species (♂)	98 (36%)	45	22	13	9	1
Species (♀) × hybrid (♂)	18 (7%)	1	1	1	0	0
Hybrid (a) (♀) × hybrid (b) (♂)	119 (44%)	17	8	4	2	1
All	271	76	42	23	15	2

4. Flowers. Of the original 271 crosses, 15 (5%) produced flowering plants.

Figure 1 illustrates the process of attrition. Two crosses created in 2014 that had not flowered as of mid-2016 (and may never do so) are shown as 'Pending'. Table 1 shows the broad classification of the 271 hybridisation attempts and their outcome. The attrition rate for the sub-set of taxa that had a parentage in sub-genus *Decaloba* was broadly similar.

Discussion

The general pattern of attrition shown in Figure 1 would probably be repeated given a different set of species and hybrids. Some crosses may result in only a few seeds being produced per fruit. Just because none of these germinate does not mean that success could not be achieved if a larger number of seeds were available. Germination rates can vary from zero to almost 100%. An example of what can happen is provided by the cross *P.* 'Wilgen K. Verhoeff' (♀) × *P. caerulea* (♂). Of around 200 seeds, just four germinated. By contrast, the cross *P. edulis* f. *edulis* (♀) × *P. caerulea* (♂) yielded less than ten seeds, but most germinated. Although more seeds from a particular cross might grow if kept for longer in moist growing medium, experience shows that if germination is ever likely to occur it will do so within 4 months.

In any given crossing attempt, the outcome can depend on the particular properties of the parent clones. Thus greater success might be achieved by using several different clones of the parent species. A good example here is shown by *P.×belotii*. This had long been thought to be sterile [12]. I had two clones, one of which I created from an original cross of *P. alata* and *P. caerulea*, while the other was from a commercial source. Neither produced seeds when backcrossed to *P. caerulea*. However, a further clone of *P. ×belotii* has now been raised and backcrossed to *P. caerulea* by Sandra Prais to produce the hybrid *P.* 'Rambam' [10].

Even when seeds germinate, failure can occur at an early stage when seedlings do not develop or produce deformed or chlorotic plants. Failure can also occur much later. For example, two crosses produced otherwise healthy plants, but flower buds either aborted or failed to open. For reasons of space, plants were discarded if no flowers formed within 2-3 years of sowing seeds. It is possible that the rate of flowering might have increased if seedlings had been allowed to grow for longer.

From **Table 1** it will be seen that there was a similar number of crosses where the male parent was a species (49%) compared to those where the male parent was a hybrid (51%). However, most successful outcomes originated from the former grouP. For comparison, of the hybrids registered from 2004 – 2010 [13] where the pollen parent was known, 90% had a species as the male parent, and only 5% had the parentage [hybrid (a) (♀) × hybrid (b) (♂)]. Although crosses with a hybrid as the pollen parent appear to be less successful at producing flowering offspring, it is likely that the most interesting hybrids in the future could result from such complex crosses. Unless many more species can be brought into cultivation, the number of simpler crosses amongst well-known species is probably approaching its limit.

Conclusions

The aesthetic properties of a cultivar are mostly subjective, but even if a novel flowering hybrid is produced, it may have little value if it is too similar to an existing hybrid, not vigorous, not free-flowering, not resistant to pests and diseases, not easily propagated from cuttings or it is malformed. It may be concluded that in trying to create a new *Passiflora* hybrid that satisfies all of the above criteria, the breeder must expect a large number of failures.

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Sizzling Sea Bass for two with passion fruit & chilli

by Myles Irvine

Ingredients

2 filleted whole sea bass
Bunch of fresh samphire
2 green chillis - mild or hot as you like.
400ml coconut milk
Coconut oil - unrefined.
Coriander leaves
2 large courgettes
Garlic bulbs - 4 cloves.
1 stick lemongrass
1 lime
3-4 passionfruit
1 heaped teaspoon crunchy peanut butter
1/2 lb sweet potatoes

Method

1. Slice the sweet potatoes and add them to a large pan of boiling water and simmer for 20 minutes. Put a steamer on top of the pan and just over half way through add the courgettes, sliced, and the samphire stalks.

2. Meanwhile, heat a tablespoon of coconut oil in a frying pan, add the sea bass and cook for two minutes at medium heat on each side then remove the fish from the pan and put to one side.

3. Top up the coconut oil in the frying pan as neccessary, chop up the garlic cloves and chillis and fry for a couple of minutes.

4. Then add the coconut milk and a little water into the frying pan, the heaped teaspoon peanut butter, the stick of lemongrass (bruise first) and the sieved juice of 3-4 passionfruit. Note: 'Ester' is an excellent tasting variety if you can get hold of it. The yellow passion fruit may need a little sugar or honey to balance their acidity.

5. Add the fish to the ingredients cooking in the frying pan and simmer gently for 15 minutes.

6. Tear up the coriander leaves at the last minute and sprinkle on top. Remove the lemongrass before serving.

7. Finally take the sweet potatoes off the heat and mash before making the plate up as shown with the fresh lime slices.