Artistic research in breeding: The Bifrost Eucalyptus project

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Abstract
Genetic signs of domestication of plants and animals date as far back as the oldest known evidence for other artistic expressions like painting, music and sculpture. Breeding is often seen as a science or a craft and is rarely considered art. The Bifrost art project aims to combine the spectacular bark and growth rate of the rainbow gum *Eucalyptus deglupta* with the cold hardiness of the cider gum *Eucalyptus gunnii* and possibly other cold-hardy species. The cold hardiness introgression should make it possible to grow amazing rainbow-colored trees in a European or North American climate. The project has been initiated and is expected to continue for decades or centuries in a distributed, participatory, manner. The project explores breeding as an art form, and through extension landscape and ecosystem manipulations that may last beyond the time when human kind has driven itself to its extinction. The project also questions commonly held beliefs about “pristine” and “natural” as being better than “artificial” and “anthropogenic”.

Keywords:
interdisciplinary, horticulture, genetics, tree, collaborative processes, evolutionary physiology, aesthetic experience, generations, land art
Background

**Breeding, domestication and the essence of culture**

The very essence of culture can be found in its etymological origins ("to grow"). We rarely think about it, but we come in contact with some of the oldest artifacts of early human activity every day through the food that we eat (domesticated and bred animals and plants) and the pets that we keep. Genetic changes through domestication of the dog from wild wolves is estimated to have originated sometime around 20,000-40,000 BCE [1], which is the approximate age range of the oldest artistic artifacts that have been found, like abstract drawings and cave paintings [2], [3], bone flutes [4] and Venus figurines [5]. An obvious difference between breeding and other kinds of art where the results often are immediate, is that the visible results from breeding typically only become apparent much later in the process. Domestication of crops happened much later (~12,000 BCE) [6], but since plants generally tolerate more extreme breeding methods and have shorter generation times (generations is a more appropriate time measure than calendar years in evolution), the genetic changes through domestication of plants is at least as advanced as those for domesticated animals. Domestication is also not a process that only happened long ago. For example, the Swedish spruce breeding project got initiated more than 150 years ago, and they are now at their second generation bred material with an estimated 10-25% yield increase [7], which demonstrates that breeding projects often have to be viewed not as the work of a single individual but as a process spanning multiple generations of breeders.Old land races which can be found in seed banks in museums also give us a historical record which not only tells us about the breeding history, but also other interesting historical facts about local needs and preferences [8-9].

Despite human kind's long experiences of breeding, it was not until the 1860s that the laws of inheritance were systematically studied by the monk Gregor Mendel [10]. In many ways, his observation and fascination inheritance of smooth and wrinkled peas could be seen as an artistic curiosity that upon systematic observations turned into a scientific study. What distinguishes "artistic research" from scientific research is as such not easily defined [11], but artistic thinking can be wider in scope and include aspects typically not considered in purely scientific studies. Artistic thinking can thereby also serve as a more indirect source of scientific inspiration. As a testament of breeding as an art form, one can only look at the phenotypic (morphotype) diversity within species that have undergone systematic and creative breeding for utility or aesthetic preferences, for example *Canis lupus* (*wolf and dog*) [12-13], *Brassica rapa* (*cabbage, brussels sprout, turnip, ...*) [14] or *Rosa spp.*(*roses*) [15].

The plant-human interaction is also in itself an interesting field of artistic research [16]. A seemingly wild environment, like a forest or individual trees, can also offer aesthetic qualities not only visually, but also through other senses [17-20]. Not only plants, but entire landscapes can also be seen as art installations, as demonstrated by land artists like Alan Sonfist. This view of living matter like plants either individually or collectively in a landscape or ecosystem ties nicely into the so-called "land art" movement. From another perspective, some conservation ("rewilding") biology efforts like the Archangel Ancient Tree Archive (www.ancienttreearchive.org) are arguably inspired by the aesthetic value of the immense redwood trees, and they are also introducing those trees to areas where they do not grow naturally which introducing an anthropogenic component. The anthropogenic influence on nature is in itself an almost baroque aesthetic quality of the contrast. For example, some historical naturalist artists like John Constable required signs of human influence for inspiration in their art. An interesting contrast when breeding and introducing new and alien species into an area is the challenge of a common will to preserve the "natural" in land art.

**Arts and life sciences**

Arts and different types of sciences will often disagree on how credits are given, which can complicate interdisciplinary collaborations [21]. Intellectual disciplines can be seen as belonging to “three cultures” [22]: natural sciences (including mathematics and engineering), political sciences
and humanities (including arts). Despite the apparent difference in nature between the natural sciences and the arts, both have a long history of mutual benefit. There are for example successful interdisciplinary collaborations where breeding has been explored as both art and science. One such example is the Cosmopolitan Chicken project [23] by the Belgian artist Koen Vanmechelen (https://www.koenvanmechelen.be/cosmopolitan-chicken-project-ccp), which evolved into the Cosmopolitan Chicken Research project (www.ccrp.be) in collaboration with scientists from Leuven university [24]. Certain forms of arts have also been invaluable for scientific study through the ages. The field of botanical art and other similar artistic illustration techniques in anatomy [25], paleontology and other fields fill a critical role since the artistic illustration of the “idea” of for example a flower often can be much more illustrative than a photography [26-27]. On the other hand is also the field of photography and by extension advanced imaging techniques critical in scientific study [28-29].

Artistic expression through breeding, mutagenesis, transgenesis or chimeras
The rules and regulations surrounding biosafety issues for genetic engineering makes do-it-yourself (DIY) “biohacking” projects using transgenic methods difficult. These rules and regulations essentially killed the kickstarter-funded “artistic science” Glowing Plant (http://www.glowingplant.com/) project [30], since the project had to resort to less efficient transformation methods in order to comply with environmental regulations. Commercial fantastic aesthetic applications of genetic engineering are however viable in some markets, for example the creation of the mythological blue rose [31], [32]. There are also some examples of purely artistic use of transgenic methods, for example the green fluorescent rabbit Alba by the Brazilian artist Eduardo Kac [33]. The intersection of transgenic methods and arts have inspired the establishment of the “vivoarts” school [34], which explores the nature of life and how ways to alter it can be used artistically but also the use of arts for non-humans. This amateur involvement in life sciences also ties nicely together with the so-called citizen science movement [35] and the “biohacking” maker movement [36].

So is genetic engineering a replacement for traditional breeding? Not always. Transgenic methods should rather be seen as a complementary tool to breeding. If the trait of interest is regulated by a single gene or a few genes, which do not need to be tightly controlled by environmental cues, transgenic methods are often superior. Many traits are however complex and regulated by many different genes that all need to be synchronized and controlled individually. In such cases (if the natural variation among sexually compatible species can provide access to the trait of interest), traditional breeding is the superior method.

For artistic reasons, it is however not always needed to alter the genome by mutagenesis, transgenesis or breeding. One alternative is to use ancient technologies to create plant chimeras using grafting, like the spectacular trees of 40 fruits from the American artist Sam van Aken (http://www.treeof40fruit.com/). As with grafting, breeding can also be done by low-tech tools which means that access to this kind of “biohacking” is open to a broader public without access to advanced laboratories.

Plants challenging the species concept
The species concept is not as clear-cut in plants as it is in animals with frequent interspecific hybridization. It is important to remember that categorizations of biological life into species, genera etc is a constructed concept, where the borders between the categories are fuzzier and less absolute than often assumed. Gene flow between species can be controlled by so-called prezygotic and postzygotic reproductive barriers. An example of a prezygotic reproductive barrier could be that while being the same species (basically a weird-looking wolf), a Great Dane will not be able to mate with a Chihuahua simply due to their size differences. This prezygotic barrier could most likely easily be overcome through artificial insemination from a Chihuahua male to a
Great Dane female, and the offspring could be bred further.

A classical example of postzygotic reproductive barriers is the sterility of the offspring (mule, hinny) between a horse and a donkey, which inhibits further breeding of the hybrid. More severe prezygotic or postzygotic barriers cause failure of fertilization or spontaneous abortion. Plants, on the other hand, can often breed across species barriers and sometimes even across genera [37-39]. There are also several manipulation techniques where post-zygotic hybridization barriers in plants can be overcome – for example through embryo rescue where embryos that otherwise would be aborted before the seed is mature can be grown in a petri dish using a growth media supplemented with nutrients and hormones [40]. This natural gene flow between distantly related species through cross breeding is challenging our classical categorizations into nice hierarchies of species and genera.

The Bifrost Eucalyptus project

Research, context and planning

In the scientific tradition, it is common to use mythological or fictional names to illustrate an object or a concept (for example celestial bodies in astronomy, genes in developmental biology). I have chosen symbolism from old Norse mythology, which nicely serves as a metaphor for the material and the aims of the project. Bifrost was the rainbow bridge between the mortal realm (Midgård) and the realm of the gods (Asgård) in Norse mythology. The rainbow bridge is a commonly used metaphor and symbol for a transition from the material and mundane to the ideal and fantastic. The rainbow also symbolizes diversity, hope, peace and LGBTQ(...) rights. The rainbow has often been used as a symbolic bridge spanning not only space but also time, for example Michael Jones McKean’s The Rainbow (www.therainbow.org). My artistic take on the rainbow starts from the rainbow gum tree Eucalyptus deglupta, an imposing (>60m) tree with striking rainbow-colored bark. The rainbow gum is unusual in that it is one of the few species of Eucalyptus growing naturally outside of Australia and in the northern hemisphere (close to the equator). Attempts at growing it outside its natural habitat are hampered by its frost sensitivity, and it can only grow in USDA hardiness zones 9 or higher [41], and the tree does not reach its full potential grandeur even in warmer climates like California due to periods of cold challenge. Until recently, the rainbow gum tree was classified as the subgenus Minutifructus, but molecular studies have placed it in the Symphyomyrtus subgenus [42] – the subgenus with the largest number of Eucalyptus species. The subgenus placement is relevant, since interspecific Eucalyptus hybrids have been successful within subgenera but not between subgenera [43]. More significantly, what was once thought to be a rare case of intersubgeneric hybridization between E. deglupta and other Symphyomyrtus eucaluptus species has already been reported [44]. Another member of the Symphyomyrtus subgenus is the cider gum Eucalyptus gunnii, which (by Eucalyptus standards) is a cold hardy plant tolerating temperatures down to between -10°C and -20°C, depending on individual variation within the species (Figure 1).

A French breeding program is using E. gunnii, and especially the extra cold-hardy endangered subspecies divaricata (Miena or “Blue Ice” cider gum) for generation of resilient and high-productivity E. gunnii X E. dalrympleana (Gundal) hybrids [45]. The snow eucalyptus Eucalyptus pauciflora has a greater cold hardiness, but belongs to another subgenus (former: Monocalyptus, now: Eucalyptus), which means that hybridization would be more difficult [43], and possibly involve advanced technologies like embryo rescue in vitro culture.

Aims
The Bifrost Eucalyptus project has three aims (Figure 2): (a) to introgress the cold hardiness from cider gum (E. gunnii) into the rainbow gum (E. deglupta), and (b) to explore the phenotypic variation in multi-generation intercrosses where equal contribution from both species is maintained – in order to combine and refine the best traits of both species. A third aim (c) is to introgress the rainbow bark trait from E. deglupta into the much smaller and harder E. gunnii.

Figure 2. A flow chart and graphical abstract outlining the breeding and selection strategy. Eucalyptus deglupta can not survive in the Belgian climate, so I rely on grafting in order to have branches survive long enough to give flowers that can be used in breeding. Similar strategies will have to be done on back-crossing breeding to E. deglupta if no collaborator in a warmer climate has been found. One major hurdle for breeding past F\textsubscript{1} will be space to grow and evaluate offspring, which hopefully will be solved by a distributed “open source” development model.

All these aims are initiated using extremely low-tech equipment and technologies (grafting \cite{46} and cut style manual pollination \cite{47-48}) in a residential setting in Merelbeke, Belgium with a hope for a future distributed development model at multiple locations.

Copyright and participatory development

Biological material can in many ways be compared to digital creations – especially for plants and other organisms that can be clonally reproduced \cite{49}, since this produces a genetically identical copy. As a consequence of this, I aim to follow an open source development model where I encourage others to either independently replicate what I am planning to do or to take material from me and breed further according to their own ideas and needs (in open source software terms: “forking” the project). This open source participatory “bazaar” model has proven to be very successful in software development \cite{50}. The copyright situation for biological material is not quite the same as for other artistic works, since it often is covered by the Plant Breeders Rights. In order to stay compatible with this system and still provide a free license which allows for further independent commercial development, I am opting for a very permissive distribution of biological material under terms similar to Creative Commons or the GNU general public license \cite{51-52}. The Open Source Seed Initiative (OSSI) share these ideals and already have a copyleft license in place which I will make use of (http://osseeds.org/) \cite{53}. Seeds and clones will be freely distributed (except distribution costs) from each generation in order to encourage the project to branch out and take unexpected paths by other people. This free distribution will also ensure that seeds and clones are challenged in a wide range of environments by enthusiasts. At later stages in the project, much larger areas will be needed for screening and selection of interesting individual plants, which can be enabled by a distributed development model.

Planned Execution phase 1: cheating Heimdall

To take the Bifrost metaphor one step further, the Norse god Heimdall (the name is believed by some to be a composite word of heim “world” and dallr “flowering trees” \cite{54}) is an appropriate symbol for the early challenges in this project – having an unusual birth (by 9 women) and being gatekeeper on the rainbow bridge Bifrost (which could symbolize pre-zygotic reproductive barriers). In contrast to crossing inbred cultivars, the phenotypic effect of crossing outbred Eucalyptus is not always predictable due to large intra-species genetic variation, which means that many F\textsubscript{1} hybrid individuals have to be phenotypically evaluated. Crossing two inbred cultivars usually has major advantages (hybrid vigor, heterosis), since it has been known for a long time that detrimental traits typically are recessive \cite{55}. The inbred Crossing two species with vastly different environmental adaptions can however lead to so-called outbreeding depression, which could either
be that the intermediate phenotype of the hybrid is mal-adapted to the intended environment or that there are genetic incompatibilities leading to inviability of the offspring (a post-zygotic hybridization barrier). Experiences with interspecific Eucalyptus hybrids for cold resistance has shown that the F\textsubscript{1} hybrids typically show an intermediate cold resistance with a slight bias towards the most sensitive parent [56]. Because of this, it is unclear if the F\textsubscript{1} hybrids will be able to survive a Belgian winter, which means that some clones will be saved in pots indoors to preserve successfully generated F\textsubscript{1} hybrids.

**Planned Execution phase 2a : Cold-hardy rainbow gum – a pot of gold at the end of the rainbow?**

If some F\textsubscript{1} hybrids can survive a Belgian winter, cold-hardy F\textsubscript{1} Bifrost clones (first generation \textit{E. gunnii} X \textit{E. deglupta}) will be back-crossed to a non-parental \textit{E. deglupta} in order to avoid inbreeding depression, and the offspring will be screened again for cold hardness. This process will be repeated iteratively until most characteristic traits of \textit{E. deglupta} are present. At that stage, independent cold-hardy backcross-lines will be intercrossed in order to attempt to further enhance the cold resistance. Intermediate generations of this breeding trajectory will be fed into the “Execution phase 2b” intercross population. If this breeding is successful, it could become an economically interesting plant for forestry applications, which means that it theoretically could be possible to get rainbow tree forests in cold climates. Imagine a cold winter morning in a forest of imposing huge rainbow-colored trees, a clear blue sky visible through the leaf ceiling, the ground covered with frost or snow. This experience alone would be an art installation.

**Planned Execution phase 2b : Advanced intercross breeding and selection – finding offspring superior to the parents through transgressive segregation?**

This breeding strategy aims to maintain approximately equal genetic contribution from both parental species and select F\textsubscript{2} and later offspring based on combinations of the best or most attractive features from both species. If F\textsubscript{1} offspring is too cold sensitive to survive a Belgian winter, intercrosses from backup clones kept indoors will be used to generate a much more diverse F\textsubscript{2} population. While cold hardiness from \textit{E. gunnii} and the rainbow bark from \textit{E. deglupta} are the two major features to select for, there are other properties like the aromatic leaves and tasty sap from \textit{E. gunnii} and the fast growth rate and impressive size of \textit{E. deglupta} that also could be selected for. A segregating population from a cross of a very cold-hardy (\textit{E. gunnii}) and a very cold-sensitive species (\textit{E. deglupta}) could also be interesting for scientific studies of the genetics of cold hardness.

**Planned Execution phase 2c : Rainbow-colored cider gum – an appropriately sized and attractive garden ornament?**

The huge size of \textit{E. deglupta} makes it a problematic plant to grow in a regular residential garden. Because of this, a smaller tree similar to \textit{E. gunnii} but with the attractive rainbow-colored bark could be a very interesting plant for ornamental purposes. If the rainbow bark is a (co-)dominant trait, cold hardy Bifrost F\textsubscript{1} hybrids will thus be back-crossed to \textit{E. gunnii} (cultivar: “azura”) to make a small and hardy rainbow-colored tree. After sufficient back-crosses, intercrosses of independent pedigrees of trees will be done to ensure the genetic diversity while maximizing the effect from the rainbow bark. If the F\textsubscript{1} hybrids do not show rainbow-colored bark, a selection of breeding material will have to be done from the F\textsubscript{2} intercross population in “Execution phase 2b” for back-crossing and subsequent inter-crossing. Material from this backcross breeding can also be fed back into the intercross populations.

**Current status**

This project does not have a well-defined start or end and will most likely span decades – if not centuries. Because of this, this public declaration of intent and description of its theoretical background is one possible starting point, which
invites other interested parties to also pursue similar aims in collaboration or independently. This art project does not care about the "who", only about the end results (the plants) which hopefully will outlive all people involved. At this moment, the *E. gunnii* parental plants are planted outside and *E. deglupta* seeds have been ordered on line and seeded. The *E. deglupta* seeds have been germinated during the winter months (January) to ensure plantlets big enough for grafting on *E. gunnii* rootstocks in the spring or early summer (April-May, when the lowest temperature is above 10°C). For technical details, see the supplemental information. If *E. deglupta* grafts are too cold-sensitive to survive the winter until flowering, I will attempt an alternative strategy: to graft buds from *E. deglupta* seedlings on adult *E. gunnii* host plants that are old enough to flower. That way, the florigen signal [57] from the *E. gunnii* host plant could induce flowering also in the *E. deglupta* grafts in the same season (June-August). If the time frame from grafting to flowering is too short, I will attempt multiple strategies to insulate the scions to allow them to grow further the next season. A clear advantage with this strategy is that we theoretically can go from seed to seed in a single year, significantly speeding up the breeding progress while reducing the need for large areas to grow full-sized trees for breeding. The cold sensitivity of the grafts could also be a simple high-throughput selection system for *F₁* and *F₂* generation plants, which would also solve the difficulties of having areas big enough for phenotypic evaluations in the offspring. Some phenotypic evaluations (for example, the rainbow bark and the size of the trees) will however require that trees are planted to grow to adulthood.

**Future perspectives and physical expositions**

This is my second “art-inspired science” project. In contrast to the first one, where I applied minimalist philosophy to the design of a small circular piece of DNA [58], this project has the potential to appeal to a much wider audience. In a way, this project is not limited in time nor space and every instance of trees grown from this project could be seen as part of a larger exposition presented at different scales – from single potted plants or trees in a garden to large forests. At a shorter-term, one aim will be to try to plant a couple of the early generations (*F₁*) Bifrost trees somewhere in the Citadelpark, Ghent, Belgium close to the modern art museum SMAK (https://smak.be/en). This site would have several symbolic values – the park is next to an actual botanical garden giving the connection to the plant sciences, the proximity to SMAK as a connection to the artistic nature of the project, and Citadelpark has a reputation to be a meeting place for the gay community [59], so the rainbow bark could also be a tribute to them.

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