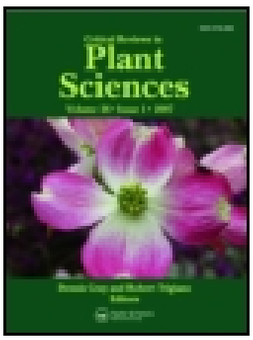

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Cannabis Domestication, Breeding History, Present-day Genetic Diversity, and Future Prospects

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ABSTRACT

Humans and the *Cannabis* plant share an intimate history spanning millennia. Humans spread *Cannabis* from its Eurasian homelands throughout much of the world, and, in concert with local climatic and human cultural parameters, created traditional landrace varieties (cultivars resulting from a combination of natural and farmer selection) with few apparent signs of domestication. *Cannabis* breeders combined populations from widely divergent geographical regions and gene pools to develop economically valuable fiber, seed, and drug cultivars, and several approaches were used with varying results. The widespread use of single plant selections in cultivar breeding, inbreeding, and the adoption of asexual reproduction for commercial drug production, reduced genetic diversity and made many present-day cultivars susceptible to pathogens and pests. The great majority of drug *Cannabis* cultivars are now completely domesticated, and thus are entirely dependent on humans for their survival. Future ramifications remain to be realized.

KEYWORDS

Afghanistan; Africa; cannabinoids; Europe; fiber; hashish; hemp; India; indica; marijuana; New World; ruderalis; sativa; seed oil; sinsemilla; terpenoids

I. *Cannabis* botany and ecology

The ecological requirements and genetic inheritance of plants determine where they grow naturally. *Cannabis* plants require well-drained soils, adequate sunlight, warmth, and moisture, so most naturally growing populations are found seasonally across accommodating northern temperate latitudes. Under natural conditions, *Cannabis* grows well along exposed riverbanks, lakesides, the margins of agricultural land, and other areas disturbed by humans (Merlin, 1972; Clarke, 1977, 1981; Clarke and Merlin, 2013; Small 2015). Based on ecological constraints, *Cannabis* evolved somewhere in temperate latitudes of the northern hemisphere, and Eurasia is favored as its primary region of origin (Clarke and Merlin, 2013). Seminal uses, early cultivation, worldwide dissemination, and eventual domestication of *Cannabis* all began within this natural biogeographical range.

Cannabis plants are usually dioecious and produce either male (pollen) flowers or female (seed) flowers. An individual *Cannabis* plant's gender is determined by X and Y chromosomes, and monoecious plants of this genus producing flowers of both sexes occur only rarely in nature. *Cannabis* also relies on air currents to spread pollen grains from male plants to female seed plants. Wind pollination, dioecious sexuality, and X/Y sexual inheritance are each relatively rare in plants, yet all three are characteristics of *Cannabis*.

Cannabis plants grow and develop within their annual life cycle of sexual reproduction. Moistened seeds germinate as spring weather warms, and juvenile plants grow rapidly through the summer. Fast-growing juvenile plants appear much alike, but as autumn day length decreases, populations begin to flower and plants express individual phenotypic differences. Male plants within a single population are often slightly taller than female plants. Male flowers hang from branches with few leaflets and are exposed to the wind (which facilitates pollen dispersal), whereas flowers on female plants are tightly clustered with small leaflets to trap male pollen grains that fertilize the female ovules. Soon after males shed their pollen they die. Before the arrival of killing frosts, the fertilized female plants ripen viable seeds. These disseminules fall to the ground via the wind or feeding birds and other animals that disperse the seeds inadvertently; and then they overwinter in the soil ready to initiate another life cycle the following spring. During their fertilized development, each enlarging seed is surrounded by a bract covered with thousands of secretory hairs called glandular trichomes. These hairs produce a resinous blend of cannabinoids (chemically related compounds found in *Cannabis*) and aromatic compounds (mostly terpene compounds common in many plants); and these secondary metabolites are believed to protect the developing seed by repelling pests and pathogens (Clarke,

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1977, 1981; McPartland *et al*., 2000). Psychoactive *delta*-9-tetrahydrocannabinol (THC) and nonpsychoactive cannabidiol (CBD) are the primary cannabinoid constituents in almost all *Cannabis*. THC and aromatic psychoactive secretions are evolutionarily significant as they attracted early human attention, and at least in modern times they remain the primary impetus for the continued breeding and worldwide dispersal of *Cannabis* cultivars (Pollan, 2001; also see Merlin, 1972; Clarke, 1998; Clarke and Merlin, 2013).

Cannabis favors a mild climate with sufficient water and sunlight, and early humans spread it into a range of favorable temperate and sub-tropical niches where it became naturalized (feral) throughout Eurasia, in parts of Africa, and more recently in the New World. *Cannabis* thrives on the nutrient-rich dump heaps near human occupation and has readily apparent agronomic traits, and it was therefore preadapted to cultivation (Anderson, 1967; Merlin, 1972; Clarke and Merlin, 2013). Annual plants of this genus branch freely when cultivated in open areas; when grown in dense stands, they suppress branching, forming a single central stalk (Iltis, 1983). This naturally adapts *Cannabis* to high density sowing for fiber production, as well as low density plantings that encourage branching and flower formation for seed and drug production.

II. *Cannabis* diversity

Environmental factors, in concert with a plant's genotype or set of genes, more or less determine its phenotype, or the visible expression of its genotype. Genetically and phenotypically diverse varieties of *Cannabis* evolved under the pressures of natural selection within the diverse environments into which they were introduced, and were further selected by humans to provide fiber, seed, or drug products. More than two centuries of studies to characterize *Cannabis* diversity and bring order to this multipurpose genus resulted in a series of taxonomic systems circumscribing one, two, or three species. Twenty-first-century taxonomic research by Karl Hillig (2004a,b, 2005) encompassed a wider geographical range and diversity of population states than previous efforts, and set the stage for understanding *Cannabis* evolution. Recently proposed taxonomic systems (McPartland *et al*., 2000; McPartland and Guy, 2004; Small, 2015) share many commonalities with Hillig's conclusions, and the evolutionary hypotheses of Clarke and Merlin (2013) are deeply rooted in his work. *Cannabis* is presently considered by many, but not all taxonomists, to be a polytypic genus consisting of two extant species *Cannabis sativa* and *Cannabis indica* each circumscribing several biotypes or subspecies (see Small, 2015 for a recent, detailed discussion of his long-held single species hypothesis). Taxonomists also recognize three population types for *Cannabis* based on natural origins and associations with humans; those that are truly wild, those that are cultivated, and feral escapes from cultivation that grow spontaneously in areas associated with and often disturbed by humans (Clarke and Merlin, 2013; Small, 2015). It is important that taxonomic systems reflect both evolutionary history and the relationships between differing gene pools. Based largely on archaeological and historical records backed by Hillig's chemotaxonomic research, Clarke and Merlin (2013) published names and acronyms circumscribing geographical and cultural groupings as an aid to understanding the roles of differing gene pools in the domestication history of *Cannabis* (see Figure 1 for acronym definitions used throughout this review.)

Northern temperate origin, annual life cycle, dioecious sexuality, camp-following and weedy tendencies, and readily observable traits of potential value (large seeds, strong fibers, and sticky sparkling resin glands) predisposed *Cannabis* to utilization, selection, and breeding by early humans. The following section of this review focuses on the impact of the human–*Cannabis* relationship upon the evolution of *Cannabis* as a crop plant (Figures 1 and 2).

III. Early domestication history

The evolutionary history of *Cannabis* and human interactions span millennia and its ongoing domestication will continue into the future. Whether early humans first used *Cannabis* as a source of fiber, food, or mind-altering compounds, they eventually developed differing agricultural techniques to increase the yield and quality of all of these products, which led to the twentieth-century breeding of *Cannabis* cultivars specifically for fiber, seed, or drug production based on local cultural preferences. Phenotypic variation between *Cannabis* populations is well documented in surveys of the wide range of cultivated fiber, seed, and drug varieties (e.g., Serebriakova, 1940; Clarke, 1981). Nature and humans have worked together to control domestication in *Cannabis*, but at times they have presented opposing forces that engendered differing outcomes. For example, isolation, artificial selection, and inbreeding imposed by humans during domestication have limited genetic diversity, whereas natural outcrossing and genome mixing have encouraged genetic diversity. The question remains: How did *Cannabis* evolve toward domestication during cultivation and breeding, as opposed to remnant wild populations and feral escapes that evolved under natural selective pressures alone? (Figure 3)

21st Century *Cannabis* Taxonomy

Cultivated *and* feral populations

Scientific name and **Primary traits**	**Biotype group**	***Origin*** and **Diffusion →**	**Common use**
C. sativa ssp. ***sativa*** Rarely psychoactive Low resin THC≤CBD	**NLH** Narrow-leaf hemp	***Europe*** → New World	**Fiber** and seed
C. indica ssp. ***chinensis*** Mildly psychoactive Moderate resin THC≈CBD	**BLH** Broad-leaf hemp	***East Asia*** → Europe → New World	**Fiber** and seed
Indica – *Wrongly called "**sativa**"*			
C. indica ssp. ***indica*** Very psychoactive High resin High THC : little CBD	**NLD** Narrow-leaf drug	***South Asia*** → Middle East → Africa → Europe → New World	**Drug**, fiber and seed
Afghan - *Wrongly called "**indica**"*			
C. indica ssp. ***afghanica*** Moderately psychoactive High resin THC≈CBD	**BLD** Broad-leaf drug	***Afghanistan*** → Europe → New World	**Drug**
"Sinsemilla" cultivars Highly psychoactive Very high resin High THC : little CBD (Some with high CBD)	**NLD/BLD** Narrow-leaf drug x Broad-leaf drug	***New World*** → Europe → Worldwide *Note: Only cultivated, no feral escapes.*	**Drug**

Figure 1. Four groups of naturally occurring *Cannabis* landraces are extant today, along with *sinsemilla* drug cultivars and industrial hemp cultivars (the latter are not shown). Presented here are proposed taxon names, acronyms representing their biotype groupings, region of origin, routes of diffusion, and primary uses.

Wild *Cannabis* populations readily yielded seed, fiber, and drugs useful to early humans. In addition to being a multi-use plant, *Cannabis* is easy to grow, and must have been included among the first plants brought into cultivation, almost certainly in the regions where people originally encountered this versatile resource. As human populations settled and expanded, they probably depleted nearby natural stands of *Cannabis*, and consequently early farmers began to grow plants closer to their homes. Soon these cultivators began to sow seeds from plants expressing traits that differed from the norm such as larger seeds, taller stalks, and/or more resin production (e.g., enhanced trichome production, increased trichome size, elevated THC content). As a result of their artificial selection, they unwittingly initiated the processes of domestication. As humans spread *Cannabis* beyond its original wild range into recently settled agricultural centers, the selective pressures of new habitats and human requirements for varying products became more evolutionarily important. Landraces are not simply the products of human selections, as natural selective pressures constantly impose their effects, but cultivation favors the outcomes of artificial human selection over those of natural selection. Farmers and breeders select plants with useful traits, resulting in different frequencies of traits (both qualitative and quantitative) between wild populations and their cultivated and increasingly domesticated descendants. Qualitative morphological changes (e.g., fruit size, stalk height, flower form and color, presence or absence of secondary metabolites, etc.) are often

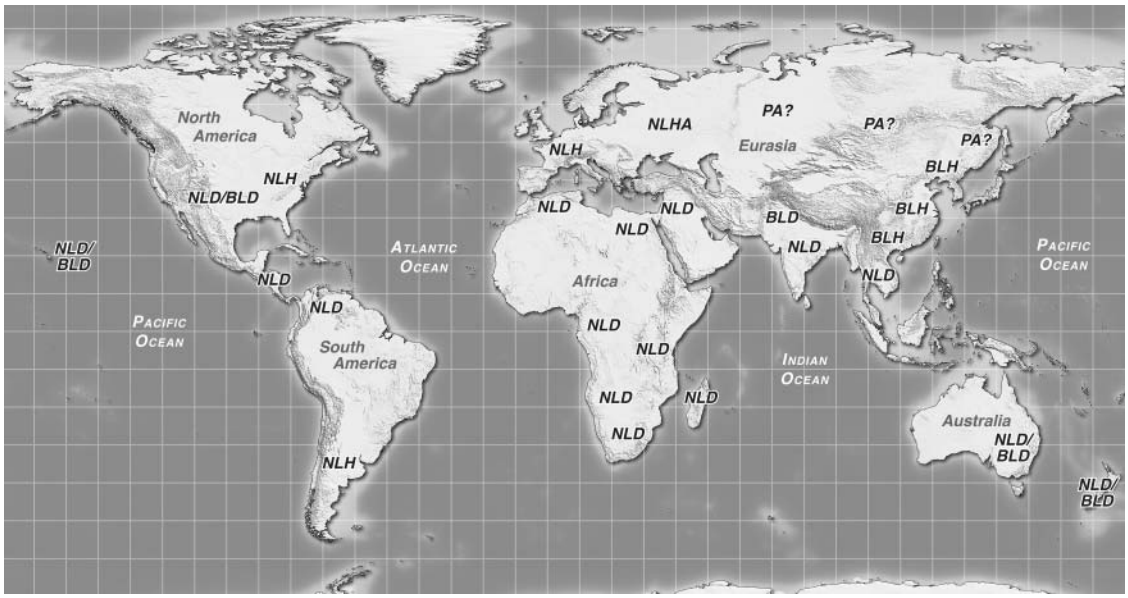

Figure 2. The various *Cannabis* taxa (indicated by their acronyms, see Figure 1) have been spread by humans into nearly every part of the world; PA? = putative *Cannabis* ancestor (from Clarke and Merlin, 2013).

Plant parts used	Use category	Material type or other benefits
Stem bark	Cordage	Long cellulose fibers
Stem fiber	Cordage and woven textiles Building materials	Long cellulose fibers Concrete reinforcement
Stems (wood and bark)	Paper	Long and short cellulose fibers
Stem wood w/o bark	Building materials Animal bedding	Chip board, concrete matrix
All parts: Primarily female flowers and seeds	Medicinal	Herbal remedies Pharmaceuticals Nutraceuticals
Female flowers and Associated resin glands	Recreational drugs	Marijuana (*ganja*) Hashish (*charas*)
Seeds Seed oil	Human food	Proteins and essential fatty acids Essential fatty acids (*omega*-3 and *omega*-6)
Seeds Seed cake Foliage	Animal feed	Proteins and essential fatty acids Proteins and trace fatty acids Vegetable mass
Seed oil	Industrial feedstock	Oil used in paint and plastic manufacture
Stem wood w/o bark Seed oil	Fuel	Heat Light
All parts: Primarily Bark Seeds and Female flowers	Ritual and social	Social activities employing various plant parts. Healing and life cycle rituals Healing and life cycle rituals Healing and inebriation
Populations	Environmental	Erosion control and CO_2 fixation
The plant, people and their interplay	Aesthetic	Intrinsic beauty of the plant
The genus	Educational	Iconic example of an economic plant and its ancient human relationships

Figure 3. Humans have found uses for nearly every part of the *Cannabis* plant and a number of traditional cultures harvested fibers, seed, and drugs from the same crop. Modern breeders developed special purpose cultivars by selecting certain plant parts (e.g., stalks, seeds, and flowers) for increased yield along with other economically valuable traits (from Clarke and Merlin, 2013).

controlled by two alleles or gene states operating at a single locus or gene position along the chromosome, and are inherited by simple dominant versus recessive Mendelian genetic mechanisms. Quantitative physiological traits (e.g., increased yield, variations in amounts of secondary metabolites, etc.) are usually controlled by a suite of alleles at several loci, their inheritance is more complex, and consequently it is more difficult to improve these traits through phenotypic selection.

Many times throughout history humans most likely carried only a few seeds to a new settlement, and often only a limited number of individual plants (sometimes only one female exhibiting specific favorable traits) were selected as parents. This produced genetic bottlenecks that gave rise to populations with restricted genomes in new areas of *Cannabis* growth. When limited numbers of seeds were dispersed into isolated regions where *Cannabis* was not already growing, and the resulting small populations were brought under new selective pressures, strong founder effects influenced the evolution of these new *Cannabis* crops and in some cases their feral escapes. Only a few plants of limited genetic diversity established new populations which eventually evolved into local landraces.

There are striking examples of founder effects in drug *Cannabis* breeding. Early-maturing Afghan broad-leaflet drug (BLD) landraces were introduced into Europe and North America in the late 1970s and early 1980s, and a handful of psychoactively potent and early-maturing individuals were selected and crossed with the narrow-leaflet drug (NLD) cultivars already growing in the West to make NLD/BLD hybrids. NLD/BLD hybrid cultivars were strongly selected for high THC content along with early maturation and large female inflorescences; this produced potent plants when grown outdoors across northern temperate latitudes, and thus revolutionized domestic marijuana production. However, the major horticultural drawback to NLD/BLD hybrids is their susceptibility to fungal infections. Their large, dense, tightly packed female inflorescences (commonly called "buds") hold moisture and make perfect microclimates for bud rot caused by the gray mold (*Botrytis cinerea* Pers.), known to fine wine makers as the "noble rot" of grapes. NLD/BLD cultivars have little natural resistance to fungal infection because the BLD founder plants were naturally adapted to the arid conditions of Afghanistan where pathogenic fungi cause little threat (McPartland *et al.*, 2000). Many NLD landraces originated from regions with relatively more humid conditions (e.g., Colombia, India, Jamaica, Thailand, etc.) and evolved a natural resistance to fungal infection. Bud rot was almost unheard of when only NLD varieties were grown, and now it accounts for significant annual agricultural loses (Figure 4).

There are no natural sterility barriers between *Cannabis* plants and crosses are generally fully fertile. Therefore, in order to reinforce and preserve the genetic integrity of the cultivar's desirable trait expressions, plants with variations in favorable economic traits must first be genetically isolated, and then continued selection and sexual reproduction must be maintained in isolation to reinforce and preserve those traits. Wind pollination between separate male and female plants favors hybridization (recombination of separate gene pools through sexual reproduction) because male and female gametes usually must come from different parents, and thus outcrossing is obligatory. In traditional cropping regimes, only seeds from favorable female plants were preserved for sowing, but the seeds on each female plant were fertilized most likely by a wide range of males, thereby restoring genetic diversity in the offspring population. Outcrossing favors dominant alleles, leading to rapid evolution of landraces and cultivars. Natural introgression (the exchange of genes between populations via a hybrid intermediate) relies on backcrossing to one (or both) of the parental populations. Plant breeding

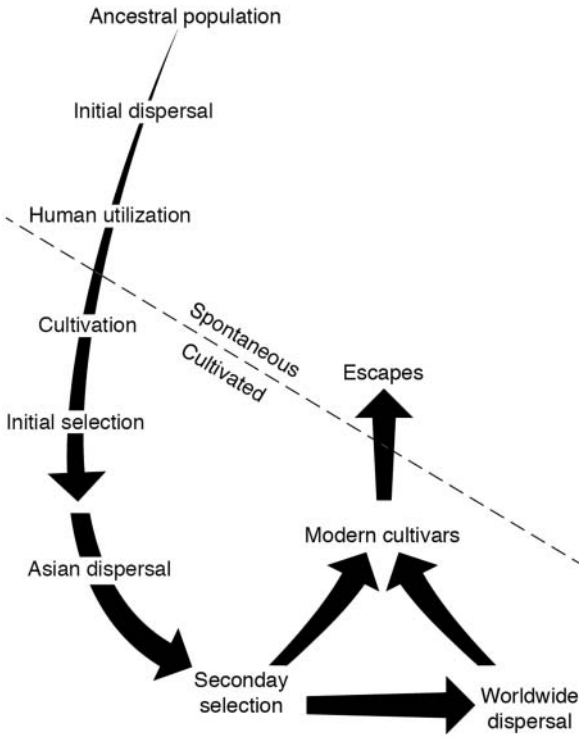

Figure 4. The domestication process starts with individual plants collected from naturally selected wild populations that are brought into cultivation, and are then increasingly subjected to human selection until they reach the cultivar stage. Some cultivars may escape human selection pressures, become feral, and return to natural selection (from Clarke and Merlin, 2013).

transfers genes between populations in the same manner, and artificially controlled introgression is the basis for breeding hybrid cultivars.

When two previously isolated varieties interbreed they form a hybrid population with more vigorous growth, a wider variety of genetic combinations than either of the parents, and unique phenotypes with conferred survival advantages that drive natural evolution. During the domestication process, hybrid offspring are artificially selected which leads to novel combinations of traits. For example, broad-leaflet hemp (BLH) landraces introduced from East Asia into Europe and North America were crossed with European narrow-leaflet hemp (NLH) landraces to create improved industrial hemp cultivars, while NLD varieties introduced into North America in the 1960s and 1970s were crossed among themselves to create improved NLD hybrid drug varieties. However, accelerated evolution occurred via artificial selection in the 1980s when domestic NLD hybrids were crossed with imported Afghan BLD landraces to create the lineages of hybrid "sinsemilla" cultivars that are widely grown today (*sin semilla* in Spanish literally means "without seed").

IV. Phenotypic changes during domestication

There are many examples of variability between cultivars selected (or not) for differing traits that changed significantly with domestication. For instance, plants of hemp fiber cultivars (both NLH and BLH) produce longer internodes and fewer branches, even when grown in open environments, whereas seed (both NLH and BLH) and drug varieties (both NLD and BLD) exhibit shorter internodes and more prolific branching even when closely sown. Traditional drug landraces produce 5.0–20.0% THC, whereas European hemp cultivars contain less than 0.3% THC (Small and Marcus, 2003). Domestication begins with the wild condition of a trait as the norm. Unidirectional or one-way evolution proceeds by artificial selection away from the wild character state in a continuum of intermediate stages leading to a domesticated form or function (e.g., from small to large seeds or from low to high content of stalk fiber); however, we only have sufficient archaeological evidence to draw firm conclusions for a few physical stages of domestication (Fleming and Clarke, 1998). Seeds are the only propagules of sexually produced landraces. Morphological changes in seed traits accompanying domestication were largely controlled by unidirectional evolution of qualitative characteristics. When early humans collected seeds for food or sowing, they unconsciously selected for nonshattering inflorescences. Persistent seeds remain on harvested plants and are taken home, whereas naturally dispersing seeds fall to the ground and are lost. Within self-sowing spontaneous populations there is no selection for nonshattering phenotypes. On the other hand, selection for nonshattering is strong in hemp cultivars because growing fiber fields requires sowing thousands of seeds, which must be harvested the previous year. Traditional Nepalese landraces are grown to produce drugs, fiber, and seed. In the case of seeds, most are eaten, with some reserved for sowing (Clarke, 2007), and nonshattering is favored in all Nepalese cultivars.

Landraces, in general, also have larger and lighter colored seeds than wild or feral populations growing nearby; in addition, the seeds of landraces also generally lack the horseshoe-shaped base and mosaic-patterned perianth associated with freely-shattering, camouflaged, wild-type seeds (Vavilov, 1931). The East Asian BLH gene pool exhibits the greatest diversity in seed coloration and size, resulting from millennia of natural and human selection within a large geographically and culturally diverse region. Chinese wild and feral seeds range in size from 100 to 500/g (approximately 2800 to 14,000/ounce), whereas large snack food seeds might have as few as 15/g (approximately 420/ounce), representing more than a twenty-fold range in seed size (Clarke and Merlin, 2013) (Figure 5).

Subtle physiological changes, although less obvious, are probably more common and possibly also more evolutionarily significant than morphological changes. In fact, increased seed size and loss of seed dehiscence characteristics are among the few morphological clues of substantial artificial selection reported; however, changes in seed physiology also accompany domestication. For example, mechanisms for perennation (inhibition of seed germination that allows seeds to survive through winter before germination occurs in the spring) are often lost. *Cannabis* farmers traditionally collect seeds and store them, ensuring their survival until the following spring, and, as a result, perennation is no longer of natural evolutionary advantage. Seeds of naturally selected feral populations evolved in response to climate fluctuations and germinate slowly and unevenly, whereas cultivar seeds are adapted to agricultural norms and must germinate quickly and uniformly. Densely sown hemp field conditions automatically select for uniform and rapid germination because late-germinating seedlings cannot compete with already established seedlings and are crowded out, thus allowing for more uniform treatment of the crop. Cultivars that have lost their dehiscence mechanisms, as well as physiological means of delaying germination, depend upon human intervention to perpetuate them—a strong sign of domestication. *Cannabis* seeds also vary widely in fatty acid content (Mölleken and Theimer, 1997) offering physiological traits for further selective breeding.

Plant part	Wild trait	Selected trait	Inheritance	Change	Crop	Selection
Fruits	Freely-shattering	Persistent	Qualitative	Morphological	F, S, D	U, U, U
	Horseshoe base	Reduced horseshoe	Qualitative	Morphological	F, S, D	U, U, U
	Fruit size: small	Fruit size: large	Qualitative	Morphological	F, S, D	U, I, U
	Fruit color: dark	Fruit color: light	Qualitative	Morphological	F, S	U, U
	Fruit coat: Mottled	Fruit coat: less mottled	Qualitative	Morphological	F, S	U, U
	Protein content: lower	Protein content: higher	Quantitative	Physiological	S	I
	Oil content: lower	Oil content: higher	Quantitative	Physiological	S	I
	Germination: delayed	Germination: rapid	Qualitative	Physiological	F, S, D	U, U, U
	Germination:staggered	Germination: uniform	Qualitative	Physiological	F, S, D	U, U, U
Stalks	Fibers: brittle	Fibers: supple	Qualitative	Morphological	F	I
	Fibers: brittle	Fibers: very brittle	Qualitative	Morphological	D	U
	Fibers: short	Fibers: long	Qualitative	Morphological	F	I
	Fiber content: low	Fiber content: high	Quantitative	Morphological	F	I
	Branching: moderate	Branching: sparse	Qualitative	Morphological	F	I
	Branching: moderate	Branching: profuse	Qualitative	Morphological	S, D	I, I
	Internodes: medium	Internodes: longer	Qualitative	Morphological	F	I
	Internodes: medium	Internodes: shorter	Qualitative	Morphological	S, D	I, I
Flowers	Fruits: few	Fruits: many	Quantitative	Morphological	S	I, I
	Bracts: few	Bracts: many	Quantitative	Morphological	S, D	I, I
	Inflorescences: few	Inflorescences: many	Quantitative	Morphological	S, D	I, I
	Resin glands: few	Resin glands: many	Quantitative	Morphological	D	I
	THC level: low	THC level: high	Quantitative	Physiological	D	I
	THC level: low	THC level: very low	Quantitative	Physiological	F, S	U, U
	Terpenoid profile: simple	Terpenoid profile: complex	Qualitative	Physiological	D	U
	Maturation: early	Maturation: late	Qualitative	Physiological	D	I
	Maturation: early	Maturation: very early	Qualitative	Physiological	F. S	I, I

Figure 5. *Cannabis* seeds, stalks, and flowers were selected for a wide number of traits that were unintentionally as well as intentionally altered during human selection and cultivar development. Quantitative and qualitative changes occur in morphological as well as physiological traits (from Clarke and Merlin, 2013). F = Fruit, S = Seed, D = Drug, U = Unintentional, I = Intentional.

Plant architecture or gross phenotypic expression has also been affected by domestication. Both NLH and BLH are selected for herbaceous unbranched stalks and long internodes associated with longer and more flexible fibers, traits expressed by both male and female plants. Hemp cultivars have also been selected for total fiber yield, and exceptional cultivars (e.g., "Kompolti TC") can produce over 40% dry weight of this product (Bócsa, 1994). Seed-propagated cultivars (NLH, BLH, NLD, and BLD), which are selected for maximum flower yield, are more profusely branched with relatively woody stalks and shorter internodes, and consequently have shorter and more brittle fibers. Artificial selection of economic traits in *Cannabis* is usually limited to females, which has had a marked effect on the evolution of the genus under domestication. However, the selection of female plants also affects the phenotypes of their male offspring. Indeed, males are heavily influenced by the selective pressures exerted on their female parent, and, during domestication, changes in male morphology (e.g., shorter height, compact inflorescences, and more flowers) paralleled changes in female morphology.

The morphology of *Cannabis* inflorescences also reflects extreme selection during domestication. Both increased seed and resin gland yields rely on increased female flower production, and both seed and drug cultivars have larger inflorescences containing more flowers than fiber cultivars, which in turn have larger inflorescences than the wild and feral types from which they originated. NLD/BLD hybrid sinsemilla cultivars were selected for mind-altering potency (e.g., higher THC percentage, increased cannabinoid synthesis, more and larger glandular trichomes) along with immense female inflorescences providing increased surface area for the

elaboration of cannabinoid-producing glandular trichomes. Because sinsemilla cultivars are reproduced asexually by vegetative cuttings, there is no need to allow space or divert energy to maturing seeds, and their inflorescences are more densely packed with flowers and small leaves than NLD and BLD landraces (Clarke and Merlin, 2013) (Figure 6).

During domestication, traits more frequently diverge from the wild condition by bidirectional or two-way evolution via disruptive selection—again resulting in a continuum of phenotypes, but with the median wild condition lying between two domesticated extremes (e.g., stalk internode length and branching pattern, variations in cannabinoid content, and timing of maturation). Disruptive selection occurs when natural selection of wild populations favors certain environmentally adaptive traits, whereas artificial selection of cultivated crops favors a certain plant product, and leads to strong evolutionary consequences during the domestication process. Variation in the timing of floral maturation offers an example of natural bi-directional evolution. Long before the appearance of modern humans, the primordial Eurasian *Cannabis* gene pools were likely divided and reformed several times during the Pleistocene as ice sheets advanced and receded across southern Europe and southeastern Asia. Differing photoperiod responses naturally evolved through geographical isolation and selection, and ecotype differences were established. Extremely early flowering, northern temperate NLH cultivars (e.g., "Finola") and much later flowering tropical NLD landraces (e.g., those from Colombia, India, and Thailand) evolved under natural selection from separate ancestral genomes that shared ancient roots in central Eurasia. In other words, these divergent landraces evolved by following individual "routes" to domestication at differing latitudes both north and south.

Another example of bidirectional evolution in *Cannabis* results from artificial selection for THC levels. Early Eurasians were presented with several non-*Cannabis* choices of alternative species producing strong fibers and nutritious foods, but relatively few other known species with psychoactive options. Revering our ancestors, creating deities, and opening cognitive channels of communication with them predate agriculture. Possibly *Cannabis* was first appreciated by early humans more for its entheogenic (e.g., spiritual perception) or other mind-altering powers, while its uses for fiber and food became more important later as settled populations grew. Only in modern times has *Cannabis* been selected for extremely low THC content. As noted above, the primary psychoactive constituent of *Cannabis* is THC, and its content varies widely between feral as well as domesticated hemp and drug populations. How did such diversity in cannabinoid content evolve? Why are fiber varieties so low in THC, whereas sinsemilla cultivars produce the most psychoactively potent marijuana the world has ever known? (Figure 7)

Feral populations, as well as hemp and hashish cultivars, that are unselected for cannabinoid content express a bell curve of CBD:THC ratios ranging from mostly

Taxon	Quantitative *vs.* Qualitative Traits	Before selection	Following selection
BLD X NLD	Quantitative	Moderate to high THC content	Very high THC content
		Moderate to low CBD content	Very low CBD content
		Low bract to leaf ratio	High bract to leaf ratio
		Low flower yield	High flower yield
	Qualitative	Moderately branched	Profusely branched
		Medium internodes	Short internodes
		Later maturing	Earlier maturing

Figure 6. Both qualitative as well as quantitative traits have been strongly selected in BLD X NLD sinsemilla hybrids (from Clarke and Merlin 2013).

THC with almost no CBD (Type I) through mostly CBD with almost no THC (Type III). However, the vast majority of hemp and hashish populations contain approximately equal amounts of CBD and THC (THC:CBD ≈ 1 or Type II), which, in the absence of human selection, may be the naturally selected and evolutionarily neutral character state of major cannabinoid production (Clarke and Merlin, 2013). Within intermediate Type II (average THC:CBD ≈ 1) landrace populations, seeds selected from the lowest THC:CBD ratio plants, as well as those seeds selected from the highest THC:CBD ratio plants, when sown in isolated gardens, will produce offspring populations with median cannabinoid ratios skewed in favor of either the high-THC (Type I) or high-CBD (Type III) parents. When these selection processes are repeated for a few generations, the following two distinct chemotypes or chemical phenotypes result: one with a narrower range of cannabinoid ratios and higher average THC content (Type I); and a second with a narrower range of cannabinoid ratios and higher average CBD content (Type III). Since psychoactive THC and terpenes are easily detected by simple human assay, early *Cannabis* farmers readily developed more potent landraces by annually selecting higher potency plants and sowing their seeds. Because CBD is not psychoactive, it can only be detected by laboratory analysis, and its levels must be increased by controlled selection and breeding. The recent public availability of analytic laboratories has revolutionized CBD breeding.

Hemp fiber cultivars are bred to produce tall, unbranched stalks; consequently, flower and seed production are relatively less important. Hemp landraces evolved in northern latitudes with a short summer growing season and long photoperiod that did not naturally favor long flowering periods and THC production. Natural selection maintained THC at relatively low levels in both East Asian BLH and European NLH landraces. Modern European NLH cultivars were artificially selected for extremely low levels of total cannabinoids, and especially lower amounts of THC. Disruptive selection increased THC and lowered CBD in drug varieties, and lowered THC production in fiber varieties, resulting in bi-directional evolution of cannabinoid phenotype over time.

Strong heritability of alleles for THC and CBD production (as well as nonproduction) allows for rapid increases or decreases in potency through selection, and there is evidence that both marijuana and hemp have been intensely selected for varying cannabinoid levels (McPartland and Guy, 2010). Inbred lines produce predominately either THC (Type I) or CBD (Type III), and crosses between pure-breeding lines produce offspring of an intermediate chemotype with approximately equal amounts of both THC and CBD (Type II); inbreeding of the F_1 intermediate generation produces F_2 offspring in a

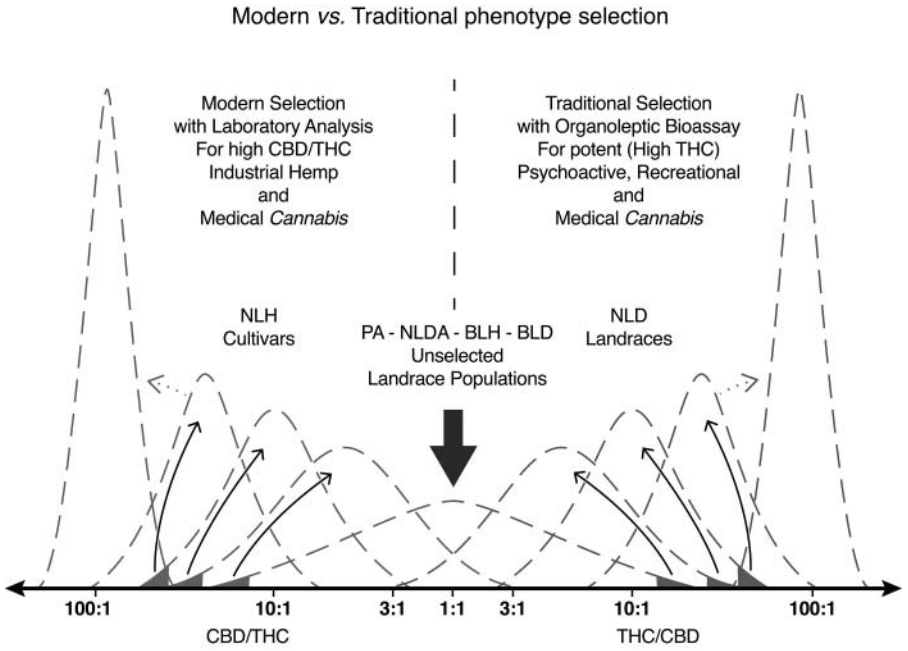

Figure 7. *Cannabis* BLH and BLD landraces are utilized for a wide range of purposes, are generally unselected for cannabinoid ratio, and synthesize approximately equal amounts (on a population average) of both CBDA and THCA. However, there are usually a few plants that produce much more THCA than CBDA, and a few others that produce much more CBDA than THCA. If the high-CBDA plants and high-THCA plants are isolated in separate breeding populations, and selection for either high-CBDA or high-THCA is continued for several generations, descendant populations will develop either high-CBDA or high-THCA profiles (from Clarke and Merlin, 2013).

simple 1:2:1 ratio including ~25% that produce nearly pure THC profiles (Type I), ~50% of intermediate cannabinoid ratio (Type II), and ~25% that produce almost entirely CBD (Type III). Marijuana-type (drug) plants inherit a highly expressed THC-acid or THCA synthase allele; hemp-type (fiber) plants inherit a highly expressed CBD-acid or CBDA synthase allele; and intermediate types inherit both active synthase genes. Mendelian phenotypic segregation in the F_2 generation indicates a pattern of inheritance where THCA and CBDA synthases are co-dominant allelic variants (B_T and B_D) at a single enzymatic locus (de Meijer *et al.*, 2003). However, subsequent genome research suggests that THCA and CBDA synthases are controlled by a number of homologous alleles at separate linked loci (Kojoma *et al.*, 2006; van Bakel *et al.*, 2011).

Recent research by Weiblen *et al.* (2015) sheds new light on the inheritance and evolution of cannabinoid biosynthesis. *Cannabis* plants synthesize the carboxylic acid derivatives of cannabinoids, THC-acid or THCA and CBD-acid or CBDA, from the same precursor molecule, cannabigerolic acid (CBG-acid or CBGA). However, CBDA synthase has a higher affinity for CBGA molecules than THCA synthase; therefore, when both synthases are active, more CBGA substrate is converted to CBDA than THCA. Low-THC hemp cultivars do have an active THCA synthase allele, but because they also have an active CBDA synthase allele they produce predominately CBD and little THC. On the other hand, high-THC marijuana drug cultivars have both an active THCA synthase allele and a *nonfunctional* CBDA synthase allele (e.g., "Skunk No. 1"), but because the CBDA synthase gene is nonfunctional there is no competition between the differing alleles for substrate, and consequently nearly all the CBGA is converted to THCA. This explains why these cultivars are high in THC with little if any CBD. Also, according to Weiblen *et al.* (2015), "the marijuana-type THCA synthase allele may be dominant over the hemp-type allele, and the functional CBDA synthase allele may be dominant over the nonfunctional allele." Heterozygosity at separate loci, gene duplication, mutation, and divergence of homologous alleles facilitated positive selection of cannabinoid synthase genes, both functional and nonfunctional (especially the nonfunctional CBDA synthase allele). This artificial selection precipitated key evolutionary steps leading toward potent high-THC marijuana. Hybrid vigor, intensive selection for more and larger glandular trichomes (Small and Naraine, 2015), allele mutation, gene duplication, and selection of potent alleles at an unlinked locus controlling cannabinoid quantity likely also played important roles in increasing the psychoactive potency of sinsemilla cultivars.

Traits apparently correlated with potency, such as later maturation, larger inflorescences, and/or increased resin production, provided clues for early humans, indicating which plants were most psychoactive. It is easy to observe which inflorescences sparkle most in sunlight and feel the stickiest, and both of these traits indicate higher resin production and potential mind-altering potency. Many times in various locations potent wild and feral plants were selected as a source of seed for sowing; this propelled differing gene pools on separate, divergent evolutionary paths upon which they remained more or less genetically isolated from other cultivated gene pools, as well as from wild and feral populations. Continued farmer selections for increased potency led to higher THC content in NLD and BLD landraces. More recently, hybridization between potent NLD and BLD landraces resulted in modern sinsemilla cultivars with increased vigor and potency.

Present-day North American and European drug cultivars are hybrid descendants of two genetically divergent drug *Cannabis* gene pools—NLD and BLD. Traditional Asian NLD landrace farmers from Thailand, India, and South Africa, as well as New World farmers in Mexico, Colombia, and Jamaica, maintained varietal purity by sampling individual plants and selecting seeds from the most flavorful and potent ones to sow the following year. Consequently, original NLD landraces imported into North America had a relatively high THC content with little if any CBD. On the other hand, imported BLD landraces from Afghanistan were relatively high in both THC and CBD. Afghan sieved hashish (a mechanically concentrated resin gland preparation) was collected from entire populations, so individual plant selection for potency was difficult and rarely practiced. As a result, cannabinoid chemotypes of individual BLD plants varied widely from producing nearly all THC to nearly all CBD, with a bell curve distribution in between focused on a median ratio of 1:1, much like unselected feral East Asian BLH populations, but with a higher total cannabinoid content resulting from selection for the readily apparent traits of larger inflorescences and more resin glands, especially on the small leaves subtending the flowers (Figure 8).

Dried sinsemilla inflorescences can contain more than 20% THC and/or CBD by weight. How did modern drug cultivars become such prolific cannabinoid producers? This unprecedented production results from a combination of simple heritability of cannabinoid profile, hybrid vigor between evolutionarily distinct and genetically distant gene pools (NLD and BLD), and subsequent, highly focused, human artificial selection for potency. Early NLD × BLD crosses expressing great vigor and diversity were used to breed seed cultivars, from which present-

Figure 8. *Cannabis* NLD and BLD plants vary in appearance throughout their growth cycles. In addition to their narrower leaflets, juvenile NLD plants are taller and more laxly branched than the shorter, more compact Afghan BLD plants (from Clarke and Merlin, 2013).

day production clones were selected. Sinsemilla cultivation provided a rigorously selective regimen resulting in cultivars with extremely high THC levels from their NLD ancestry, and little if any CBD from their BLD ancestry. Seed-propagated drug cultivars must be rigorously selected to maintain their potency. Feral drug *Cannabis* populations lose potency quickly because open pollination does not impose selective pressures favoring high THC content, low CBD content, or favorable aromatic profiles. The atavistic lowering of potency in naturalized populations may indicate that THC is not of great natural adaptive significance. Apparently, enhanced THC synthesis is primarily an artifact of human selection, and represents another key aspect of the ancient human–*Cannabis* relationship (Clarke and Merlin, 2013).

Natural evolutionary determinants were important before humans started using and spreading *Cannabis*, and natural selection will always play a role in its evolution. Domestication may have had more profound effects on the evolution of the functional physiology of *Cannabis* rather than its anatomical physical traits, and perhaps plants of this genus were so well preadapted for agriculture that few morphological changes were required. Nevertheless, human selection during domestication has had by far the greatest influence on changes in *Cannabis* phenotypes, both morphological and physiological. This process accelerated during the last half of the twentieth century as industrial hemp and marijuana breeders developed new cultivars through vigorous artificial selection.

V. Twentieth-century *cannabis* breeding

Cannabis produces copious quantities of both pollen and seed, yet it is not an easy plant to improve by selective breeding. *Cannabis* populations are almost always dioecious, with male and female flowers occurring on separate plants which are therefore normally incapable of selfing (self-fertilizing). Selfing is the most effective sexually reproductive means of fixing desirable traits, because selected genes are more likely to be represented in both the male pollen and the female ovule if they come from the same plant. In traditional *Cannabis* breeding, recessive alleles controlling a selected trait locus (aa) must be present in two separate individuals, one male or pollen parent (either Aa, aA, or aa) and one female or seed parent (also either Aa, aA, or aa). As a result, in open-pollinated outcrossers such as *Cannabis*, qualitative traits are usually controlled at single loci by dominant allelic forms (AA, Aa, and aA), which are much more common than recessives (aa) (3:1 ratio) and heritability is high. Quantitative traits are more often controlled by differing allelic forms at various loci; as a result, heritability is low, which makes improvement by breeding more difficult. Female plants supply most of *Cannabis*' economically valuable products, including fibers, seeds, or drugs, whereas male plants merely fertilize the females and are occasionally harvested for their fiber. This makes it difficult for plant breeders to recognize potentially favorable traits in a male parent, as these traits must ultimately be expressed in female offspring. All *Cannabis* plants are wind-pollinated, which allows them to intercross freely; therefore, in order to avoid random fertilization and seed set, selected female seed parents must be isolated from males until they are to be pollinated by a selected male parent. Successful breeding of open-pollinated cultivars requires the identification of plants with favorable traits, and then creating breeding lines via recurrent selection, making hybrid crosses between these lines, and field testing their progeny (Posselt, 2010).

It is a costly and lengthy process to develop true-breeding *Cannabis* seed cultivars. In addition to a high degree of planning, attention, and patience, a breeder needs organization, infrastructure, and stability to pursue crop improvement goals; and then must successfully maintain commercial cultivars through continued selection of elite lines. Large population sizes are required in order to select very few individual breeding plants with favorable combinations of economically interesting characteristics. Much of recent drug *Cannabis* domestication has taken place secretly without legal sanctions, using small populations with a high percentage of select survivors. Under these circumstances, clandestine breeders still made lucky crosses; however, progress is much faster with more plants to choose from, more focused selection criteria, and lower selection percentages. Commercial drug *Cannabis* seeds have been widely available for more than 30 years, but continued improvements relied on the many hobby breeders who made interesting crosses and distributed the seeds to other breeders. Although breeders operated on limited scales and in near secrecy, the sum of their creations overshadows their individual roles; indeed, their cumulative efforts and early crowd sourcing laid the foundations for the plethora of asexually reproduced sinsemilla cultivars available today.

Sexually reproducing and outcrossing *Cannabis* may (by the nature of its genome randomizing reproductive system) be resistant to morphological changes associated with domestication in other crop plants. Until the recent popularity of asexual propagation to ensure the perpetuation of consistently high-yielding, high-potency drug crops, *Cannabis* cultivars had not become completely domesticated, but now the asexual sinsemilla varieties survive entirely at the discretion of humans. The discussion that follows documents the twentieth-century breeding of fiber, seed, and drug cultivars; this is where we outline the pedigree and lineage of example cultivars, emphasizing the roles of individual plants from diverse gene pools in cultivar domestication and evolution via human selection.

VI. Industrial hemp breeding

European NLH landraces were introduced to the New World by sixteenth- and seventeenth-century colonists, and eventually they became naturalized to a broad range of local climates and soil regimes. Even though fiber yields of the early introductions were relatively low, they were well-suited for seed production, and were the only hemp grown in North America until the early 1890s. During the late nineteenth and early twentieth centuries, Japanese hemp was introduced into California, and Chinese hemp into Kentucky. Both of these early introductions from East Asia (likely broad-leaflet hemp, i.e., BLH varieties) were from a gene pool that evolved independently from European NLH varieties. The Japanese introductions were lost, whereas descendants of Chinese introductions into Kentucky became known as "Kentucky" hemp. By the turn of the twentieth century, the United States Department of Agriculture (USDA) hemp breeding experiments revealed that the introduced Chinese varieties would lose their favorable characteristics of high fiber quality and yield when randomly reproduced by farmers. Soon after seed production was purposefully restricted to only a few isolated farms under the attention of plant breeders, the commercial "Kentucky" cultivar became relatively uniform and genetically stable (Boyce, 1900). Later hybrid crosses involved the 'Ferrara' cultivar from Italy (possibly an NLH/BLH hybrid), 'Kentucky' hemp (NLH?/BLH), and additional Chinese BLH landraces. Subsequently, further selections were made from these complex hybrids to produce even higher fiber yield. Sadly, no known North American hemp cultivars remain alive today, only their feral offspring.

During the twentieth century, European hemp breeders improved locally available, native landraces and also made crosses with alien imports to create higher yielding fiber and seed varieties. It is evident from their breeding histories and chemotaxonomic profiles that many present-day European cultivars share common ancestors (de Meijer, 1995) and are less genetically diverse (Hillig, 2004a) than their related feral populations and landraces. Crosses between select individuals from NLH landraces belonging to the Mediterranean and Central Russian ecotype groups (Serebriakova, 1940) formed the basic breeding lines of European hemp cultivars. Brief summaries of their individual and shared breeding histories are provided below: these outline the breeding strategy employed, with an emphasis on the interactions of differing gene pools and the effects of individual plant selections. For a more detailed discussion of European hemp cultivars, see de Meijer (1995) and Clarke and Merlin (2013).

Open-pollinated, outcrossing plants like *Cannabis* are naturally heterozygous having a higher frequency of differing alleles at the gene loci, especially for many dominant traits (Aa and aA); however, they lose vigor when they are inbred to achieve homozygosity with a higher frequency of identical allele pairs at recessive gene loci (AA and aa). The primary objective of breeders is to "fix" selected traits through homozygosity while maintaining overall heterozygosity and hybrid vigor in the cultivar. There are several techniques used to breed *Cannabis* cultivars. Early hemp and drug landraces were developed through recurrent mass selection. Farmers

favor homozygosity by sowing seed collected each year from plants with agriculturally valuable traits, whereas natural selection for health and vigor encourages heterozygosity in the remaining traits. Mass selection is most effective in improving simple qualitative characteristics with high heritability, although persistent recurrent selection can lead to changes in more complex quantitative characters with relatively low heritability. Mass selection is used effectively in hemp breeding to enhance highly heritable traits such as fiber and cannabinoid content (Hennink, 1994).

Mass selections or phenotypic selections are usually made by choosing the best plants in the field before harvest, and saving their seeds for sowing. Both mass family and single plant selections are made to produce more genetically homogenous lines for breeding. This is essentially how local feral populations become landraces. Individual female plants are selected, but a single female plant is usually fertilized by a random assortment of many unselected male plants, and, as a result, most of the offspring are half siblings, rather than full siblings. In an outcrossing plant such as *Cannabis*, mass selection is more efficiently imposed by identifying superior male plants in a population prior to pollen dehiscence, destroying all males that do not fit selection criteria, and then collecting seed only from select females. This was how the dioecious European hemp cultivars were selected and maintained (e.g., "Carmagnola," "Kompolti," "Lovrin," and "Novisadska"). Recently, Chinese hemp cultivars (e.g., "YunMa 1" and "YunMa 5") were mass selected from local Yunnan province landraces (Salentijn *et al.*, 2014).

As scientific breeding progressed, monoecious hemp cultivars based on the "Fibrimon" cultivar were developed by crossbreeding selected individuals from different landraces and cultivars to increase variability and vigor. Crossbreeding to combine desirable traits is achieved by fertilizing a favorable female plant with pollen from a single favorable male plant. The seeds are grown and the F_1 population is open-pollinated so each female is fertilized by many males. Each selected F_1 female is evaluated by growing out her offspring (F_2), and favorable lines are continued (F_2, F_3, …) by further half-sibling selections. A select female can also be fertilized by only one select male from each population and then offspring will be full siblings. This promotes homozygosity while further narrowing genetic variability in the offspring. Single crosses between a female and a male parent were also used to establish most sinsemilla cultivar lineages (see below).

Monoecious plants with male and female flowers, and sub-dioecious female plants with male flowers, allow self-fertilization and inbreeding. Monoecious individuals were used to breed monoecious hemp cultivars (e.g., "Fibrimon") and female unisexual cultivars (e.g., "Uniko-B"). Female unisex cultivars could serve a similar purpose as male-sterile lines if they produce no viable pollen, have very high seed yields, and can be used to facilitate hybrid seed multiplication. Recessive traits are fixed by serially selfing sub-*dioecious* females, and vigor is restored by combining two or more lines in the final hybrid cultivar (de Meijer, 2004). Hybrid single cannabinoid medical cultivars have been developed by the following methods: (1) selecting individual female plants containing the target cannabinoid (or other compound) from several genetically diverse origins; (2) using hormonal stimulants to form pollen-bearing male flowers on each female plant; (3) selfing each female line in isolation for several generations (S_1, S_2, S_3, …) to encourage homozygosity and "fix" production of the target compound; and finally, (4) crossing genetically less related individual inbred lines to restore vigor in the hybrid cultivar (de Meijer, 2014).

The majority of present-day European hemp cultivars are monoecious. The French cultivar "Fibrimon" was selected directly from an inbred monoecious line created from a selfed, individual plant with both male and female flowers (monoecious or sub-dioecious) that occurred spontaneously in a NLH population cultivated in Central Russia. Pseudo-monoecious cultivars were developed by crossing monoecious "Fibrimon" with dioecious female high-fiber German NLH selections (also originally from Central Russia), or with late-flowering NLH landraces from Italy or Turkey, and then backcrossing to "Fibrimon." Genetic homogeneity of monoecious cultivars must be maintained each year by careful selection of monoecious parents and the creation of elite monoecious seed. Second-generation crops sown from elite seed consist almost entirely of monoecious plants, and are multiplied by open pollination to produce commercial sowing seed for fiber cropping. Third-generation fiber crops include up to 20% true male and female individuals resulting from inbreeding and natural genetic drift in the absence of human selection. Traditional dioecious landraces were easily maintained by local farmers who produced their own seed, but today farmers must purchase sowing seed each year because monoecious cultivars immediately decline without careful selection and breeding (de Meijer, 1995); this creates a market situation similar to seed production of hybrid maize (or corn, *Zea mays*).

Although the traditional Italian fiber cultivar "Carmagnola" and its descendants "Fibranova," "Eletta Campana," and "Superfibra" are practically unavailable today, the Italian gene pool formed an important modern foundation for both European and American hemp breeding. East Asian BLH landrace varieties that were brought to

Italy early on were responsible for much of the heterosis effect (hybrid vigor) sought by European hemp breeders. All of the Italian cultivars were dioecious, and most are now believed extinct. Open-pollinated "Carmagnola" was of great antiquity, having originated from a Chinese BLH landrace, and it formed the basis of Italy's famed high-quality hemp textile production. "Carmagnola Selezionata" was selected directly from "Carmagnola," while 'Fibranova' and 'Eletta Campana' resulted from crosses between traditional 'Carmagnola' and high-fiber German strains obtained from the Central Russian NLH landraces that were also used in breeding 'Fibrimon.'

Traditional Hungarian landraces and cultivars are mostly dioecious. For example, "Kompolti" was selected for high fiber content from an Italian variety likely related to "Carmagnola." Intentional heterosis breeding resulted in several hybrid cultivars. "Uniko-B" is the progeny of "Kompolti" crossed with monoecious "Fibrimon," and first-generation crops of this cross consist of nearly all female plants, resulting in very high seed yields. Unisexual female lines produce almost no pollen and can be used for seed parents in hybrid seed production. Second-generation commercial fiber and seed crops produce approximately 30% males and still have a relatively high seed yield. "Kompolti Hibrid TC" is a three-way-cross hybrid in which two selections of Chinese origin (BLH), one dioecious and the other monoecious, were crossed to produce a unisexual, almost purely female hybrid known as "Kinai Uniszex." In the second generation, the unisex line is used as the female parent in the crossing of "Kinai Uniszex" × "Kompolti" cultivars. This hybrid produces commercial sowing seed of the triple-cross (predominately BLH) fiber cultivar "Kompolti Hibrid TC" with a restored 50% female to 50% male sex ratio, hybrid vigor, and increased fiber yield. "Kompolti Sárgaszárú" is a dioecious, chlorophyll-deficient, yellow-stemmed Hungarian paper pulp cultivar obtained from a cross between "Kompolti" and a yellow-stemmed mutant offspring from a cross between Finnish early-maturing NLH and Italian late-maturing hemp (possibly an NLH/BLH hybrid); this cross was then repeatedly backcrossed with "Kompolti" to transfer (introgress) the yellow-stemmed trait to homozygosity in the "Kompolti" cultivar (Figure 9).

Polish hemp cultivars are monoecious. For example, 'Bialobrzeskie' resulted from serial crossings of dioecious NLH cultivars of predominately Russian origin that were finally crossed with 'Fibrimon,' followed by long-term single line selections for high fiber content. Another Polish hemp cultivar, "Beniko," was obtained by individual progeny selection from a "Fibrimon" hybrid.

Romanian "Fibramulta 151" is a dioecious selection from a single cross between "ICAR 42–118" and German "Fibridia." The parent variety of "ICAR 42–118" was the progeny of an Italian "Carmagnola" × "Bologna" (another Italian cultivar) hybrid crossed with the Turkish "Kastamonu" landrace. The dioecious "Lovrin 110" cultivar was bred by selection among family groups from the Bulgarian "Silistrenski" NLH landrace. Monoecious "Secuieni 1" was derived from the crossing of "Dneprovskaya 4" × "Fibrimon," followed by two semi-backcrosses with "Fibrimon 21" and "Fibrimon 24,"

Figure 9. Four fiber hemp cultivars can be visually distinguished in this field trial. The Hungarian cultivar "Kompolti Sárgaszárú" or "yellow-stemmed Kompolti" is a reduced stem chlorophyll variety that was bred as feedstock for the paper industry (from Clarke and Merlin, 2013).

respectively. The Russian dioecious parent "Dneprovskaya 4" was a descendant from Italian hemp.

Eight cultivars are presently grown in central and southern parts of Ukraine and several regions of Russia, where the history of traditional and commercial hemp fiber and seed cultivation is very lengthy. Hemp cultivars from the former USSR are classified into maturity groups or geographical types (Serebriakova, 1940; de Meijer, 1995). Current cultivars belong to either the southern, later-maturing group or to hybrid progenies from an earlier-maturing central group crossed with later-maturing southern hemp; hybrid cultivars of the latter group are intended for sowing in higher latitudes of central and northern Europe than those ecologically preadapted to southern Europe. The dioecious southern Russian cultivar "Kuban" was obtained by ten cycles of family group selection in the hybrid population by the crossing of "Szegedi 9" × "Krasnodarskaya 56" cultivars. "Szegedi 9" was selected in Hungary from the native "Tiborszállási" landrace (NLH) and crossed with "Krasnodarskaya 56," which probably came from a cross between a local Caucasian (NLH) landrace and an Italian cultivar. The two other cultivars cultivated in areas of Ukraine and Russia are "Zenica," a dioecious southwestern Eurasian cultivar, and the NLH landrace "Ermakovskaya Mestnaya," which is cultivated on a significant scale in Siberia and belongs to the Central Russian maturity group (Serebriakova, 1940).

The remaining Russian and Ukrainian monoecious cultivars exhibit a southern, later-maturing growth pattern, but are also cultivated at higher latitudes to promote a longer vegetative growth period, resulting in taller stalks and increased fiber yield. These cultivars include "USO-11" bred from three parental populations, "Dneprovskaya 4," "USO-21," and "Dneprovskaya Odnodomnaya 6." "USO-11" is presently grown in Canada and New Zealand for oil seed production. The remaining Ukrainian cultivars were produced by crossing various Russian landrace accessions with cultivars originating in Italy and France (de Meijer, 1995).

"Finola" is an early-maturing Finnish variety used for seed production in more northern latitudes and was registered in the European Union (EU) in 1999. It is grown extensively in Canada for seed and seed oil production (Small and Marcus, 2002). "Finola" was selected from the pooled seed of two nearly identical, early-ripening, far northern Russian NLH landraces originally obtained from the Vavilov Research Institute (VIR) in St. Petersburg, Russia. The Canadian seed variety "Anka" was also selected from VIR accessions. "Finola" matures early and provides both high seed yields (up to 1.7 metric tons per hectare or 1500 pounds per acre) and short straw (stalks); in addition, it can be harvested by combine harvesters for both fiber and seed yields from the same crop (Weightman and Kindred, 2005).

European hemp breeders developed more uniform monoecious varieties with increased resistance to various pests (McPartland *et al.*, 2000), while also focusing on further reducing already negligible levels of THC. The French cultivar "Santhica" was selected from a single plant with no THC and high CBG content; and it may lack the THC synthase gene (B_T allele) (de Meijer, 2004), but it has not been released commercially. Although research institutes might develop new hemp cultivars for specific uses, government organizations and/or seed companies continue to reproduce and sell sowing seed of existing varieties and traditional farmers continue to propagate their landrace seed. As long as hemp remains an economically viable albeit minor crop, hemp cultivars will be multiplied; however, as soon as economic interest wanes, they will most likely become extinct.

Present-day European industrial hemp cultivars share common ancestors. Most fiber cultivars originated from "Carmagnola," a traditional hybrid Chinese-Italian BLH/NLH dioecious cultivar that was combined with various Central Russian NLH landraces. On the other hand, while nearly all monoecious varieties are descendants from French 'Fibrimon,' a single, inbred, monoecious cultivar that was also derived from a Central Russian landrace. Genes of the modern industrial hemp cultivars came from two taxonomically distinct *Cannabis* groups, European NLH and Asian BLH, but via only a handful of individual parents and hybrid crosses. Hemp cultivars are maintained as select elite populations, which ensures inbreeding to preserve favorable traits; some outcrossing is allowed to encourage health and vigor, but all within a heavily selected and fundamentally restricted genome preserved by intensive artificial selection.

Early in the twentieth century, Chinese BLH landraces were incorporated into North American hybrid hemp cultivars, and were later used by European breeders to make hybrid crosses with NLH landraces of European origin. The high degree of genetic difference between East Asian BLH and European NLH gene pools was illustrated by Hillig (2004a) who placed them in subspecies of different species—*C. indica* ssp. *chinensis* and *C. sativa* ssp. *sativa*, respectively. The heterosis effect or hybrid vigor resulting from crossing plants of diverse genotypes was important in the development of all modern fiber cultivars. As we will see below, heterosis breeding was also a key factor in the initial development of present-day drug cultivars.

VII. Sinsemilla cultivars

Early humans repeatedly introduced NLD landraces that originated in southern Asia into unusual geographical regions. Many of these introductions evolved into new, local

landraces that eventually (sometimes centuries later) entered the international market. In the 1960s and early 1970s, all commercial marijuana imported into North America and Europe owed its heritage to the vast NLD group, and seeds of many different landraces were available in relatively large amounts. Seeds from Colombian and Thai marijuana grown at more nearly equatorial latitudes rarely produced plants that matured to the floral stage when cultivated outdoors at temperate latitudes before cold autumn weather killed them, whereas northern Mexican and Jamaican varieties often matured earlier, before harsh winter weather set in. The domesticated NLD hybrid varieties of the early and mid-1970s originated largely from crosses between Mexican or Jamaican landraces and more potent, but later ripening, Colombian, Indian, Panamanian, and/or Thai landraces (Figure 10).

Traditional Asian and New World drug *Cannabis* cultures favored and selected potent landrace varieties that in turn provided North American and European breeders with the basic genetic building blocks for developing sinsemilla varieties. In the early 1970s, gardeners began to grow sinsemilla marijuana by removing all male plants from their fields, leaving only the unfertilized (therefore seedless) female plants awaiting pollination, each producing thousands of floral bracts covered by a myriad of resin glands. Sinsemilla cultivation also brought growers to the realization that a few select male plants could be isolated from which they could collect pollen to intentionally fertilize flowers on a few female branches. This in turn produced seeds of known parentage which essentially gave birth to the intentional breeding of potent hybrid drug *Cannabis*. Early clandestine breeders combined and recombined imported NLD landraces in multi-hybrid crosses, and by 1980 North American sinsemilla ranked among the world's most potent drug cannabis.

Traditionally, both hemp and drug cultivars were developed by variations on the theme of mass selection, but modern sinsemilla cultivars evolved in a different way from hemp cultivars. Sinsemilla cultivars were usually developed by crossing a single male of one genetically distinct landrace with a single female of another landrace (e.g., Mexican NLD × Colombian NLD) to create an F_1 hybrid. In the subsequent F_1 generation, selected male or female offspring were bred by following one of three basic pathways: (1) they were inbred with one or more siblings (e.g., Mexican/Colombian × Mexican/Colombian) to establish a heterozygous and relatively inconsistent F_2 population hybrid used for subsequent mass selection to increase homozygosity and uniformity; (2) they were backcrossed with an "aunt" (seed parent) or "uncle" (pollen parent) (e.g., Mexican/Colombian × Colombian) to increase homozygosity and enhance specific traits before establishing a backcross population hybrid used for subsequent mass selection; (3) they were outcrossed with an unrelated plant (e.g., Mexican/Colombian × Thai) to create a new F_1 hybrid serial generation. Strategies one and two were followed by some early sinsemilla breeders, but pathway three was by far the most frequently pursued. By introducing a new exotic landrace or hybrid parent into the breeding line (e.g., [Mexican/Colombian × Thai] × Jamaican landrace), heterozygosity and diversity were increased by creating a new F_1 hybrid in each serial breeding generation. Although the offspring looked less and less like any

Figure 10. Narrow-leaflet drug (NLD) type *Cannabis*, closely related to this late-nineteenth-century Indian *ganja*, was spread from South Asia through Africa and eventually to the New World (from Clarke and Merlin, 2013).

of their increasingly distant ancestors, a level of relative homogeneity between siblings persisted because in each new generation half of the gene pool was contributed by a single parent. Although serial hybridization was not an intentional breeding strategy, the heterozygosity breeders unwittingly created a cultivation process that has played an important role in the continued survival of the incestuously recombined drug *Cannabis* gene pool (Figure 11).

In all three paths of drug cultivar breeding described above, landraces were crossed to make hybrid populations. However, no true hybrid seed varieties were created because no pure-breeding lines were maintained to use each year as parents of the F_1 hybrids. Only more recently have sinsemilla seed companies multiplied true hybrids by preserving asexually reproduced vegetative clones of select male and female parent plants. However, the clonal parental lines are often highly heterozygous, and their "hybrid" offspring are frequently inconsistent phenotypically.

Sinsemilla breeders have selected primarily for stronger potency (higher THC content) as well as complex aromas and flavors, which are all traits related to terpeno-phenolic secondary product metabolism in the glandular trichomes (e.g., cannabinoids and terpenoids).

Figure 11. North American and European marijuana growers crossed NLD varieties from Mexico (above) with NLD varieties from many other locations (e.g., Colombia, Jamaica, Thailand, and India), and these became the first hybrid "home-grown" *sinsemilla* cultivars (from Clarke and Merlin, 2013).

Initial serial hybridization followed by careful inbreeding (pathway 1, described above) and/or backcrossing (pathway 2) of hybrid crosses resulted in some of the early NLD varieties that are still popular today (e.g., "Original Haze," "Big Sur Holy Weed," etc.). During the late 1970s, many clandestine marijuana breeders successfully developed early-maturing and high-yielding, potent, aromatic and colorful connoisseur NLD varieties, and as a consequence demand for high-quality sinsemilla grew. Soon an exotic new series of cultivar introductions would dramatically change the face of sinsemilla breeding and the evolution of domesticated drug *Cannabis*.

VIII. Introduction of the Afghan cultivars

Broad-leaflet drug (BLD) seeds were introduced from Afghanistan into North America and Europe several times from the late 1970s through the 1980s. BLD plants were very distinctive in appearance; being well-branched and standing only one to two meters (about three to six feet) tall at maturity, they were shorter and bushier than NLD plants with broader, darker green leaflets. After BLD varieties were introduced in the late 1970s, growers commonly began to call the original NLD varieties "sativas" because their tall growth and narrower, lighter green leaflets more closely resembled NLH fiber varieties than they did the more recently introduced shorter and darker green Afghan BLD hashish varieties commonly called "indicas." The authors now assume that NLD and BLD populations belong to different subspecies of *C. indica*, subspecies *indica* (NLD) and subspecies *afghanica* (BLD).

Afghan BLD plants mature earlier than most NLD cultivars at northern temperate latitudes (from late August through September) providing an abundant source of highly psychoactive resin traditionally used to make Afghan hashish (Clarke, 1998). Seedless BLD flowers smelled and tasted much like potent hashish, and connoisseurs paid premium prices for such an exotic contraband. Breeders crossed BLD varieties with their sweet-tasting, but later-maturing, NLD varieties to produce early-maturing BLD × NLD hybrids, commonly called "indica/sativa" hybrids (in our view, hybrids of different subspecies of *C. indica*). Hybrid vigor was strong, and flower yields increased, yet plants rarely exceeded 3 m (10 feet) in height. Afghan × Thai hybrids proved particularly pungent and extremely potent. By the mid-1980s, the vast majority of sinsemilla in North America owed a portion of its heritage to the BLD gene pool. Thereafter, it became increasingly difficult to find either pure NLD (pre-BLD) varieties or the pure BLD introductions popularized only a few years earlier. Australian and Pacific Island outdoor varieties were often based on

Southeast Asian landraces introduced in the 1970s, and until more recently were less affected by BLD/NLH introductions and indoor surreptitious cultivation (Figure 12).

During the 1970s and 1980s, relatively early in the evolution of modern-day sinsemilla cultivars, NLD seeds inadvertently imported in illicit marijuana were far more commonly sown than artificially selected hybrids produced by domestic growers. Even after the introduction of Afghan cultivars, rare, intentionally bred seeds were sometimes passed from one breeder to another, but their distribution was quite limited. During the mid-1980s, seed companies began to distribute selected varieties, but accidentally produced seeds from illicit commercial sinsemilla were still much more commonly grown. Seeds carrying varying proportions of the NLD and BLD gene pools were grown and crossed repeatedly without conscious selection; this inadvertently produced multi-cross hybrids in which culturally favorable traits were rarely selected and thus the average psychoactive and aromatic qualities of domestic marijuana decreased.

Most of the offspring derived from these crosses were dissimilar to their parents and their siblings, in both appearance and potency because their genomes consisted of small random pieces of genetic information inherited from assorted predecessors, and undesirable characteristics that previously had been suppressed through careful selection were often expressed (e.g., lower potency, dull psychoactive effects, acrid aroma, harsh taste, etc.). Many of the early NLD/BLD cultivars offered hardy growth, rapid maturation, and tolerance to cold, allowing sinsemilla to be grown outdoors in many northern temperate climates. This revolutionized the domestic marijuana market both geographically and culturally by widening the scope and popularity of outdoor sinsemilla cultivation. NLD/BLD hybrids not only changed Western outdoor growing, but their rapid maturation time, short stature, and high yields also preadapted them to indoor growing as well. Tall and late-maturing NLD cultivars were poorly suited for artificial light growing, but shorter and faster ripening NLD/BLD hybrids were ideal for commercial indoor production. The combination of increased prohibition pressure, improved lighting systems, asexually propagated indoor cultivars, and high marijuana prices fueled the rapid, very widespread strategy of cultivating sinsemilla indoors in many regions of the world. The rampant success of this agricultural model narrowed the selection criteria of breeders toward developing only indoor cultivars, further limiting the present-day genetic diversity of drug *Cannabis*. Although consumers and commercial growers largely accepted NLD/BLD hybrids, serious sinsemilla breeders began to view them with more skepticism, and started to develop new drug cultivars for a wider variety of applications (Figure 13).

Figure 12. Broad-leaflet drug (BLD) Afghan hashish cultivars were introduced into Western Europe and North America in the late 1970s, and hybrid crosses with NLD cultivars changed domestic drug *Cannabis* production forever. This large BLD plant is growing in a family garden in northern Afghanistan (from Clarke and Merlin, 2013).

IX. Present status of the *Cannabis* genome

As a result of millennia of dissemination and contact with differing human cultures worldwide, *Cannabis* plants, through natural and artificial selection, have evolved huge genetic diversity which is expressed in a remarkably wide range of phenotypes. Traditional farmers in geographically isolated regions selected and maintained genetically unique landraces that formed the foundation for breeding modern hybrids. The *Cannabis* gene pool is amazingly diverse; however, breeders' well-intentioned domestication efforts have been strongly affected by the repercussions of prohibition and consequently its genetic diversity is increasingly being attenuated.

The vast majority of European industrial hemp cultivars are based on the same few single plant selections; and the drug *Cannabis* gene pool has reached a genetic bottleneck caused by incest breeding between close relatives, intentional and unintentional selfing, highly directed selection for drug potency, and the preponderance of vegetatively reproduced commercial populations. The genetic diversity of *Cannabis* is constantly reduced by persistent law enforcement seizures, accidental

Figure 13. Female *Cannabis* flowers produce a preponderance of glandular trichomes or resin glands that secrete a cannabinoid- and terpene-rich essential oil. Selection for high production of resin glands and essential oil is the driving force in modern-day drug *Cannabis* breeding, to the virtual exclusion of other agronomically valuable traits (Photo by Todd McCormick from Clarke and Merlin, 2013).

agricultural mishaps, and the gradual culling of economically unfavorable clonal lines; commercially valuable clones are propagated, and the remainders are destroyed. If we project present trends into the future, genetic diversity will continue to decrease, the lack of sexual recombination will lower the potential for evolving pest resistance, and susceptibility to agricultural pests and diseases will continue to increase. When pathogenic organisms such as viruses, fungi, mites, and aphids attack a genetically uniform, asexually reproduced crop, losses are often extensive. Illegal drug *Cannabis* cultivation and breeding have historically suffered from similar problematic pest infestations that hindered and often overwhelmed the successful cultivation of other monocrops of commercially valuable plant species.

Devastating agricultural blights have indeed caused great economic losses in several different crops, and are directly associated with diminished genetic variability leading to susceptibility to a pest or pathogen. Susceptibility to pathogens can arise in two ways. When a cultivar is introduced to a new region it may be infected by introduced alien organisms for which it has no naturally evolved resistance (e.g., Afghan BLD cultivars parasitized by gray mold in humid regions of North America). Alien pests have also been introduced into new regions where *Cannabis* crops have no natural resistance (e.g., the broomrape parasite introduced to Kentucky from China where it caused widespread damage to hemp crops, and hemp russet mites introduced to Indiana along with South Asian seed stocks). McPartland *et al.* (2000) provides more detailed histories of *Cannabis* pests and diseases.

History presents us with several different serious blight scenarios. The mid-nineteenth-century Great Famine of northern Europe was caused by a virulent strain of *Phytophthora infestans*, a parasitic and nonphotosynthetic fungus-like organism, which is closely related to brown algae; *P. infestans* commonly infects crop plants, but not *Cannabis* (McPartland *et al.*, 2000). The virulent HERB-1 strain of *P. infestans*, that causes the potato blight, originated in Mexico; it subsequently spread into North America where it caused widespread damage to potato crops, and then, in 1844, it traveled by ship to Europe where the infection was rapidly dispersed. Regions where a wider variety of potato cultivars were being cultivated were not as severely affected, but the majority of potatoes in Ireland were of the "Irish Lumper" variety, which proved to be particularly susceptible. The Irish potato crop loss in 1845 is estimated at between one- third and one-half of cultivated acreage, and in the next year three quarters were lost to blight. Nearly a million people starved to death, and another two million emigrated, many to the United States. Potato cultivars are asexually multiplied by dividing tubers, so every offspring is genetically identical to its parent plant, and therefore all share the same susceptibility to pests and diseases. Periodic failures of the northern European potato crop persisted until the early twentieth century when breeders produced potato cultivars resistant to HERB-1. Drug *Cannabis* clones are asexually multiplied by cuttings, and like potato cultivars all the plants within each clone are equally susceptible to pests and diseases, which, as we well know, spread rapidly in monoculture crops (Kelly, 2013).

During the mid-nineteenth century, the Great Wine Blight affected many vineyards and devastated the European wine industry. During the third quarter of the nineteenth century more than 40% of French vineyards were destroyed, and the suffering economy also accounted for increased emigration to North America. The blight was caused by the phylloxera aphid (*Daktulosphaira vitifoliae*) that originated in eastern North America, where it was a well-known grape (*Vitis*) root parasite. North American *Vitis* rootstocks had been introduced to Europe years before, but they did not carry the pest. In the late 1850s, phylloxera-infested *Vitis* rootstocks were transported across the Atlantic by steamship, allowing the pest to reach Europe alive, where it spread rapidly

through European vineyards of traditional *Vitis vinifera* wine cultivars that had not evolved natural immunity to a North American pest. Grape breeders crossed susceptible European wine cultivars with phylloxera-resistant native North American species, but the offspring were not particularly resistant to phylloxera and they made poor wine. The solution lay in reintroducing phylloxera-resistant rootstocks from North America (*Vitis aestivalis* or other American native species) and replacing the susceptible *V. vinifera* rootstocks. Following considerable resistance by traditional French wineries, endangered French wine grape scions were successfully grafted onto rootstocks of resistant North American species and the European wine industry was resurrected (Ordish, 1987).

Traditionally, the vine plants of table and wine grapes have been vegetatively propagated by grafting shoots, much the way modern sinsemilla varieties are propagated by rooting cuttings. A recent genetic survey by Myles *et al.* (2010) of grape accessions in the USDA collection came to the following conclusion: "We propose that the adoption of vegetative propagation was a double-edged sword: Although it provided a benefit by ensuring true breeding cultivars, it also discouraged the generation of unique cultivars through crosses. The grape currently faces severe pathogen pressures, and the long-term sustainability of the grape and wine industries will rely on the exploitation of the grape's tremendous natural genetic diversity."

In the early 1900s, the American chestnut blight, caused by a wind-borne fungus (*Cryphonectria parasitica*), decimated American chestnut tree forests before eventually spreading to Europe. This aggressive fungus arrived in North America with infected Chinese ornamental chestnut trees that were resistant to the disease. The blight is spread by wind-borne pathogenic spores that moved the fungus quickly through dense native chestnut stands, eventually killing an estimated 40 billion trees; every chestnut tree in the contiguous forests of the eastern United States succumbed to the disease in less than 40 years (Freinkel, 2007). The only survivors were apparently resistant trees or those in small outlying populations that the disease did not reach. Subsequently, an isolated, disease-resistant chestnut tree from Ohio that had survived was crossed with three naturally resistant Chinese cultivars. The resulting hybrids remained disease free, but were shorter than the average American chestnut trees. Breeding efforts continued by backcrossing the hybrid offspring three times to the American chestnut to transfer the genes for disease resistance to a cultivar with the taller American chestnut phenotype.

Southern corn leaf blight (SCLB) is a fungal disease found in many of the world's maize-growing areas. This blight is caused by *Bipolaris maydis*, which occurs in three genetically variant races. Race T infects maize plants with Texas male-sterile cytoplasm (Tcms), which contains a gene causing susceptibility to the race T fungus. Maize plants are monoecious and self-pollinating; and before this susceptibility was clearly understood, breeders encouraged a disastrous fungus invasion by unwitting selection of maize plants infected with Tems cytoplasm.

During the traditional multiplication of hybrid maize seed, farmers remove the male flowers (tassels) from the female seed plants by hand so they will not self-fertilize, and therefore will only receive pollen from monoecious plants grown in the same field. Removing tassels is time-consuming and as a result it provides summer employment for students, but it is costly and prone to human error; consequently, in the early 1960s, breeders developed lines with Tcms to be used as male-sterile seed parents in hybrid maize seed production. Hybrid sowing seed rapidly gained popularity, and by the late 1960s an estimated 90% of the American crop contained Tcms cytoplasm, making it extremely vulnerable to SCLB. By 1970, SCLB reached epidemic proportions destroying 15% of America's corn production with losses estimated at one billion dollars. The SCLB epidemic occurred because female seed lines carried an unknown male-linked trait that conferred disease susceptibility to a huge percentage of the crop. As a result, farmers returned to detasseling hybrid seed crops until breeders selected SCLB resistant cultivars and found sources of male sterility other than Tcms (Agrios, 2005). Male-sterile seed lines could be used in monoecious hemp breeding, and growing cuttings of sterile high-THC females would guarantee that drug crops would remain seedless wherever they are grown, offering another advantage to sinsemilla growers.

In 1890, a wilting disease of banana was observed in the "Gros Michel" plantation crops of Costa Rica and Panama that reached epidemic levels throughout the early 1900s. This precipitated catastrophic damage, forcing banana plantations to move to new regions where the disease had not yet spread. In 1910, the causal agent of "Panama disease" in Cuban bananas was linked to the soilborne wilt fungus, *Fusarium oxysporum* f. spp. *cubense* (Foc wilt). *Cannabis* is also susceptible to closely related *Fusarium* wilts (McPartland *et al.*, 2000). The entire dessert banana trade was based on the vegetatively reproduced "Gros Michel" (Big Mike) cultivar that had no resistance to the Foc wilt, and by the 1960s was almost entirely wiped-out. A search for banana varieties resistant to Foc wilt revealed specimens from botanical gardens in the United Kingdom and the United Fruit collection in Honduras. These were vegetatively reproduced by tissue culture, and by the late 1960s two closely related "Cavendish" clones, "Dwarf Cavendish" and

"Grand Nain," were widely spread throughout the commercial banana producing areas of Central America and beyond. Presently, these two clones account for nearly 50% of global banana production (http://www.promusa.org/Tropical+race+4+-+TR4). Also in the 1960s, Panama disease was first noticed in Taiwanese "Cavendish" bananas. Subsequently, the Panama disease became a very widespread and serious problem in various areas of the world; it destroyed Taiwan's banana industry, spread through southern China, wiped out plantations in Indonesia and Malaysia, threatens the Australian and Philippine industries, and has also been identified in Middle Eastern and East African plantations. Unfortunately, Panama disease spreads easily and potentially endangers monoculture banana production everywhere. Regrettably, the banana industry has been slow to respond to the renewed threat of Panama disease, and presently there is no market replacement for the "Cavendish" cultivars. Another pest infestation threatening plantations of bananas is the virulent Foc wilt strain known as Tropical Race 4 (TR4) that was not identified until 1994. TR4 is now spreading rapidly, infecting susceptible plantations of "Cavendish" clones, all of which are asexually reproduced and have no chance to develop resistance to TR4 (http://www.promusa.org/Tropical+race+4+-+TR4).

The blights described briefly above were caused by different pests and diseases affecting various wild and cultivated plant species, but their rampant spread relied on one key factor—the underlying cause of all these epidemics has been a lack of genetic diversity. The "Irish Lumper" potato was an asexually reproduced clone; French varietal wine grapes shared common ancestors and the roots of all were susceptible to phylloxera; genetically similar American chestnut trees formed huge contiguous forests that all perished; the American corn blight infected all hybrid maize cultivars that shared Tcms cytoplasm; and "Cavendish" bananas are all members of two closely related clones. These historically documented disasters highlight the need to further understand the diversity and present status of the *Cannabis* genome, and should give us pause for thought.

By the dawn of the twenty-first century, it was apparent that Dutch sinsemilla varieties were predominantly based on three founding Pacific coast varieties: "Northern Lights," "Skunk No. 1," and "Haze" (Clarke, 2001). Dutch, British, and Spanish companies are the largest suppliers of drug *Cannabis* seeds today, mostly selling multi-hybrid combinations of "old" varieties. In the twenty-first century, many "new" varieties have been incorporated into the modern collective gene pool; however, many of them include reassorted alleles from the seminal *Cannabis* varieties distributed far and wide 30 years ago. What appear as novel combinations today reflect a breakdown of suites of characteristic landrace traits, and the persistent reshuffling of individual landrace genes in modern multi-hybrid cultivars. Over many generations, sinsemilla cultivars have become hybridized to the point of inbreeding and are increasingly similar genetically to one another.

Because *Cannabis* can be a difficult plant in which to fix traits through selective breeding, and only female plants are of economic importance for drug production, asexual reproduction or "cloning" by rooting vegetative female cuttings provides an easy solution for rapidly expanding commercial sinsemilla production. Under this female vegetative planting regime, there are no male plants to remove, the crop can be treated uniformly, all the plants ripen at the same time, and they can be harvested together. If the electricity grid does not fail, grow rooms can produce three to four crops per year, and yield over 450 g (one pound) of dry flowers per square meter (about 10 square feet) each harvest. Male plants in breeding programs can also be preserved in a vegetative "library" under long photoperiod and induced to flower when pollen is required. Cloning radically changed *Cannabis* agriculture (and selective breeding pressures) by making sinsemilla growing possible and profitable for hobbyists. However, in one generation, asexual cloning fixes traits forever (apart from artificially or naturally induced mutations) and attenuates the genetic diversity promoted by sexual seed reproduction (Figure 14).

Reliance on only a few select male and female cuttings for seed production reduces genetic diversity. The number of female clones used as seed parents in sinsemilla breeding is now limited, but even fewer pollen parents are used to make commercial seed. Seed varieties and clones are most often named after their female seed parent, but equally important is what plant was used as a pollen parent. Male parents that consistently produce uniform offspring are much harder to find than female parents, which are often maintained as commercial cultivars. Individual male plants are often selected for phenotypic traits resembling females (e.g., increased vigor, short internodes, profuse branching, dense inflorescences, strong aroma, etc.). However, in order to identify which male clone will produce the best offspring, each of these male clones must be crossed with a range of female cuttings. The seeds from each of these crosses must then be progeny-tested to check for quality and consistency. For example, if ten prospective male clones are each crossed with ten individual female clones, and 200 seeds (approximately 100 will be female) of each test cross are sown, about 10,000 female plants will be produced, and then they must be screened to determine which male and female clone combinations produced the best offspring.

Figure 14. Uniform modern-day drug *Cannabis* crops are grown from transplanted vegetative and asexually reproduced cuttings—either inside under lights, in greenhouses, or outdoors under the sun—on various scales (from Clarke and Merlin, 2013).

A brief summary of the breeding of "Skunk No. 1" will illustrate what is involved in developing a relatively true-breeding seed cultivar. Several plants were grown from a local California "skunk" variety, which was likely a $^{3}/_{4}$ NLD and $^{1}/_{4}$ BLD hybrid (e.g., Colombian/Afghan × Mexican) and then all the females were crossed with a single select male. A female identified as skunk plant number one was selected as the highest yielding and most potent, and became the founder of all subsequent generations. For the following nine growing seasons, at least one branch of each of the (up to 100) female plants was fertilized by pollen from a male selected from the offspring of the previous year's best female plant. Seeds from the select females were sown the following spring. After only two or three generations, "Skunk No. 1" was relatively homogenous and true-breeding compared to other hybrid lines that continued to segregate in the F_3 and F_4 generations, and "Skunk No. 1" was then deemed ready for large-scale selection of parent plants with specific combining ability (SCA) for subsequent breeding.

A total of nearly twenty thousand plants were grown in a common garden from the seeds of ten females from the most promising lines selected from the previous generation, and the ten best females from among all those lines were selected based on vigor, potency, type of effect, flower yield, high flower to leaf ratio, resin gland development, amount of branching and pest resistance, as well as attractive floral aromas and flavors. Ten males were also selected for their vigor, pest resistance, female-type growth form, and aroma. All 20 selections (male and female) were reproduced asexually to preserve their unique genotypes.

Ten clonal copies were made of each of the ten select females. Pollen was collected from each of the ten select male clones, and pollen from a single male was used to fertilize a single copy of each female clone, resulting in 100 individual crosses. Two hundred seeds of each cross were sown (20,000 total) and approximately 100 female progeny of each cross were evaluated for phenotypic consistency (homozygosity) of their favorable agronomic traits (see above). Five female and three male clones were selected and used for "Skunk No. 1" sowing seed multiplication as well as hybrid cultivar development (Figure 15).

Few sinsemilla seed companies invest as much time and money in selecting superior male plants as they do for specially selected females. Most simply select a male plant based on visual characteristics alone without any chemical analyses, and cross that individual male with each of their female clones to produce commercial hybrid seed. These seeds (all containing genes from a single male parent) are then widely distributed and grown to maturity, and from which female cuttings are then selected for commercial sinsemilla production. Commercial *Cannabis* seed production can be compared figuratively with historical human conquests. A dominant invading male defeats all his rivals, procreates with select captive women, selects one son as his chosen heir, and marries his choicest daughters off to distant lands; thereby ensuring his genetic dominance at home, while spreading his genes far and wide.

More recently, "all-female" cultivars have been produced by transforming a female plant (XX) with hormone applications to produce male flowers with viable female (XX) pollen. The offspring of female plants fertilized with female pollen are all female. Every seed produces a useful female plant and there is no need to cull male (XY) plants, which provides the advantages of asexual propagation, but in the convenience of a seed. Female seeds lower genetic diversity for the same reason as female cuttings, because there are no male plants, and therefore sexual reproduction and recombination are all but impossible without making hybrids. Furthermore, although "all-female" seeds are sexually reproduced, only a few female plants can successfully be transformed into high-yielding pollen parents. Often, only one transformed female pollen parent is used to fertilize seeds on all the recipient females, and thus all of the "all-female" offspring share the genes from that single transformed

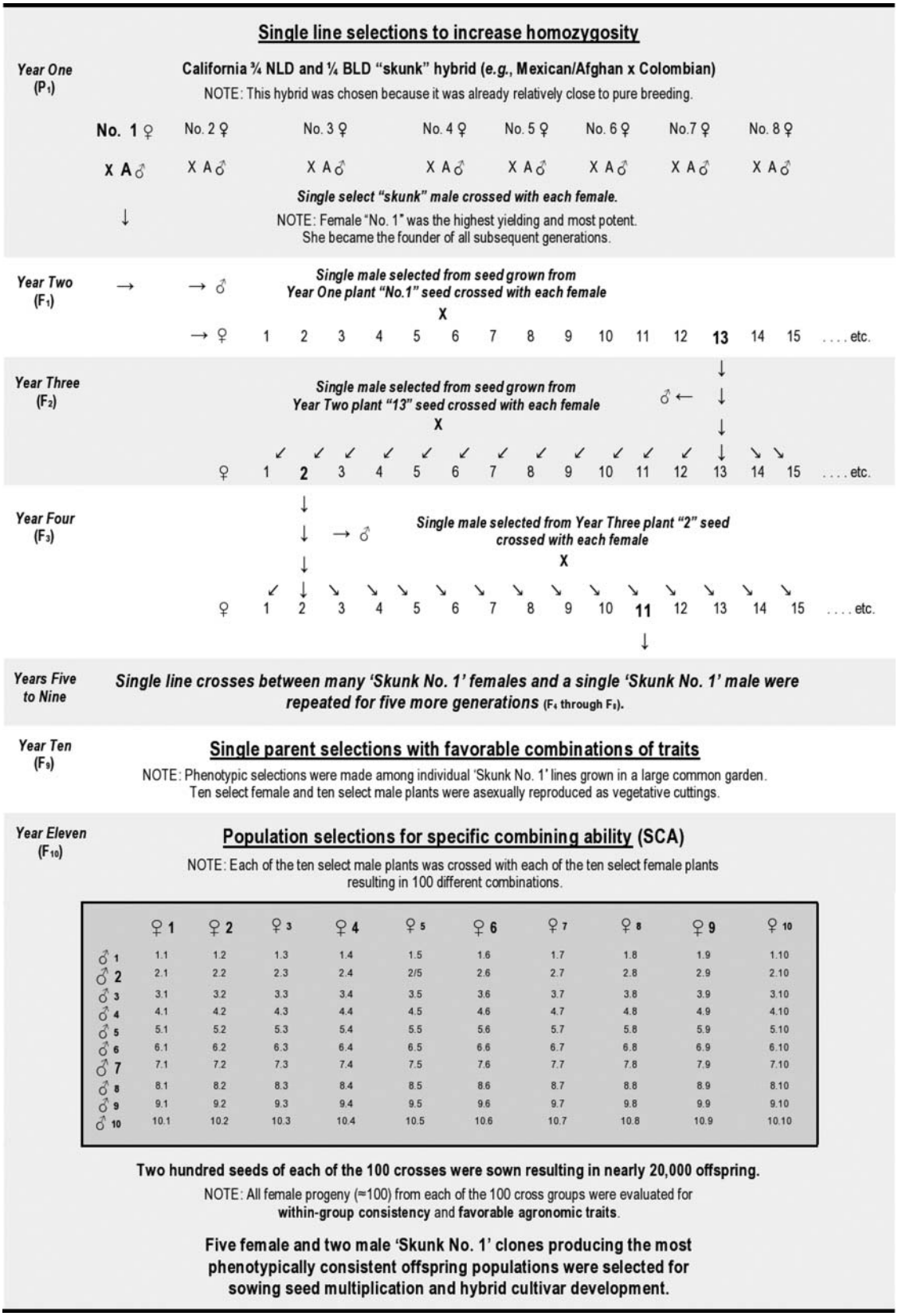

Figure 15. "Skunk No. 1" was among the first nearly true-breeding NLD/BLD hybrid drug cultivars developed, beginning in the 1970s. The "Skunk No. 1" single line was selected and bred through nine reproductive cycles to increase homozygosity and the resulting uniformity. This was followed by selection in a common garden of individual breeding parents (ten female and ten male) that were asexually reproduced before being intercrossed, and their offspring were later grown out and evaluated to determine their specific combining ability (SCA). Five female and three male plants were preserved for further seed production.

pollen parent. For example, in Spain, a single "White Widow" NLD/BLD hybrid cutting that transforms easily (as well as reliably producing all female offspring) is used to pollinate almost all of the individual female NLD/BLD clones for which "all-female" F_1 hybrid seed cultivars are named. However, several seed companies also produce single-selfed (S_1) cultivars by crossing each transformed female plant with other members of the same female clone, with no apparent loss in vigor.

All-female cultivars have gained popularity in hobby and indoor growing markets, and are also increasingly used in commercial cannabis production regions. Well-intentioned Westerners took modern NLD/BLD seeds to Morocco in the 1980s and crossed their progeny with the traditional Rif Mountain *kif* or local marijuana landrace. Within ten years, traits from the "improved" Western varieties were seen in nearly all the Rif populations. Presently, all-female cultivars are increasingly popular in Morocco. The females are completely fertilized by male plants growing nearby and the "better" improved seed is sown the next year. In this way local landraces are quickly swamped out by invasive genes, and are therefore replaced by an unselected crossbred mix of Western varieties that are poorly adapted to local environmental conditions and processing methods. Mexico, Jamaica, and Thailand have also lost many of their landrace populations (Figure 16).

In addition to "all-female" cultivars, breeders have developed "auto-flowering" cultivars that commence flowering when they reach an early stage in vegetative development rather than waiting for decreasing day length to trigger flowering. In addition, all modern "auto-flowering" cultivars likely have a common ancestor, possibly the "Finola" NLH seed cultivar or another variety which flowers independent of photoperiod constraints. Now these two traits are combined in "all-female/auto-flowering" cultivars. Although originating from two diverse gene pools (NLD/BLD and NLH), these cultivars are presently reproduced using only a few parents. Auto-flowering plants cannot be maintained as

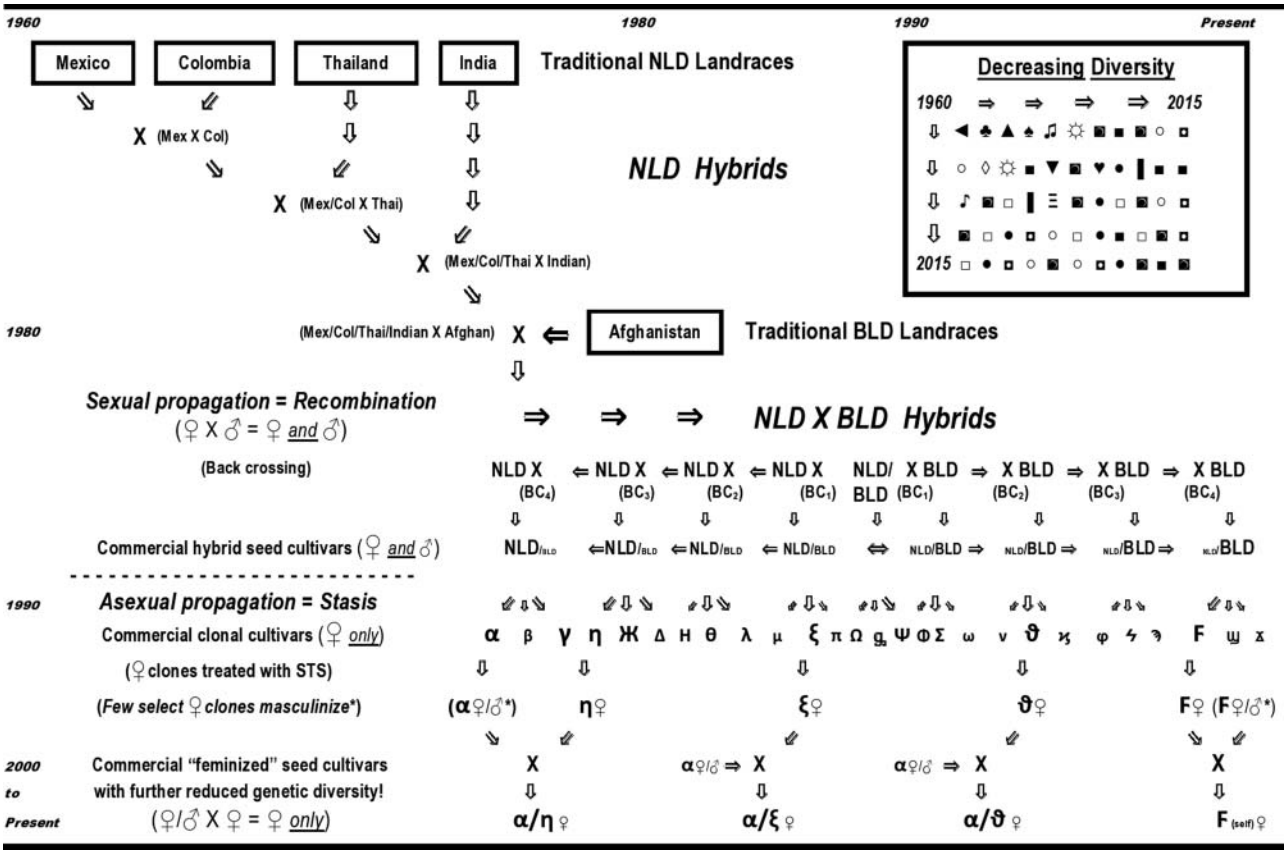

Figure 16. During the 1960s and 1970s, NLD landraces from diverse geographical regions which contained widely differing genetic combinations were combined to form hybrid NLD *sinsemilla* cultivars. In the 1980s, several BLD landraces from Afghanistan were introduced and thus added more diversity to the *sinsemilla* gene pool. By the 1990s, asexual propagation was commonly used to capture heterozygous genotypes for use as commercial clonal cultivars. The widespread (yet narrowly focused) search for cultivars with unique aromas and effects, combined with the proliferation of asexually reproduced cutting populations, obviated the need for sexual seed reproduction, but also lowered the genetic diversity of drug *Cannabis* as a whole. Recently, inbreeding to produce all-female and auto-flowering seed lines has further restricted genetic diversity.

clones because they will flower and die even under long day length, but could possibly be maintained *in vitro* and regenerated into explants.

In the past decade, many novel cultivars have appeared, and, with the exception of variations in aromatic terpenoid profiles, they are largely very similar in the remainder of their phenotypic traits and agronomic attributes. This has occurred not only because of the incessant and thorough reshuffling of a diverse, yet limited, set of genes and their respective alleles; more recently the homogenization of cultivars and their traits has been produced by accidental incest between closely related siblings or cousins. Many popular "female" clonal cultivars will produce a few functional male flowers when they are stressed by any of a wide variety of factors that are not far outside of optimum growing conditions (e.g., changes in temperature, overabundance or shortage of nutrients, fluctuations in light cycles, etc.). The male flowers of these popular "female" clonal cultivars produce viable pollen and result in unintentional fertilization of the neighboring "female" plants. Unstable female sexuality becomes reinforced generation after generation because plants with unstable sexuality are the pollen parents. Usually only a few accidentally produced seeds occur in sinsemilla (because it is usually seedless). However, among these, a high percentage will be sprouted, most will be "female," and they will strongly resemble other popular cultivars, but with their own slight differences; consequently, the accidentally produced seeds that do mature are likely to be brought into cultivation.

Along with the unfavorable tendency to produce male flowers and unwanted seed, modern varieties have some other drawbacks: they are increasingly finicky about growing conditions, are susceptible to pests (e.g., various mites) and disease (e.g., gray mold and powdery mildew), and are generally difficult to grow. Increasing numbers of pests and diseases lead to increasing use of unapproved pesticides and plant growth regulators, which can directly impact public health.

Without open-pollinated sexual reproduction, there is much less chance for evolutionary change. In the past, as early agriculturalists spread across new geographical frontiers, they exposed *Cannabis* to new and evolutionarily challenging environments. The tremendous genetic diversity of geographically isolated wild populations and traditional landraces of *Cannabis* produced by sexual reproduction and genetic recombination presented novel phenotypes for natural and human selection. Narrow selection and strong founder effects have seriously attenuated genetic diversity, bringing us closer to potential disaster. A final blow may come from commercialization of a small number of select varieties that satisfy limited consumer preferences. Under the assumption of eventual legalization, and modern monocropping paradigms favored by "economically prudent" strategies to maximize profits, fewer clonal cultivars will pass the consumer filter, and it is likely that only these will be proliferated. Variety registration to protect breeder's rights will favor asexually reproduced clones because it is easier to demonstrate uniqueness, uniformity, and reproducibility with clones than it is by breeding consistent seed cultivars. Given the present-day economic and political climates, and the increasing trend toward asexual reproduction in both indoor and outdoor crops, this scenario seems probable. If this outcome comes to pass, it will further narrow the *Cannabis* gene pool to include only the most commercially viable cultivars, and therefore, more of less repeating the history of varietal wine grape selections and potentially leading to another major crop disaster.

Whether varieties are lost through prohibition, custodial neglect, or economic priorities, they become extinct and are potentially gone forever. When indigenous farmers maintain localized landraces, they are preserving genetic diversity because landrace populations reproduce sexually, and thus allow for genetic recombination, mutation, and evolution under human selection. The numbers and ranges of traditional agricultural societies are diminishing worldwide, and many landraces are already extinct, which does not bode well for the future of the *Cannabis* gene pool. The loss of any genetic diversity threatens unique genes and allelic combinations, as well as lowering future evolutionary fitness for the genus as a whole and limiting the diversity of desirable products that crop plants may be able to provide.

The status of the worldwide *Cannabis* genome has changed dramatically since the middle of the twentieth century. The majority of traditional fiber, seed, and drug landraces are no longer grown, and many of the few remaining in situ landraces have been genetically diluted through interbreeding with introduced modern cultivars. Also, European industrial hemp cultivars share much of the same germplasm—dioecious cultivars were bred by crossing middle Russian and Chinese landraces, and monoecious cultivars all share "Fibrimon" as a pollen source—and overall the genetic diversity of NLH is currently very limited (Hillig, 2005a,b). Commercial sinsemilla cultivars are asexually reproduced, and almost all share varying amounts of both NLD and BLD heritage. Founder effects from BLD landrace selections introduced gray mold and powdery mildew susceptibility that cause millions of dollars in crop losses annually. Novel gene combinations still arise because the modern drug *Cannabis* gene pool is based traditional landraces from geographical origins that represent two genetically distinct primary gene pool, NLD and BLD. Whenever a seed is planted, it represents the sexual union of two parents, and

creates an opportunity for new genetic combinations to appear. Asexually reproduced cuttings are potentially genetic dead ends—only sexual reproduction offers us a truly brighter genetic future and the possibility for plant improvement.

X. Seed banks

When a *Cannabis* landrace is not reproduced every five to ten years, the stored seeds will most likely die and the landrace may be gone forever. Seeds must be properly kept in a gene bank and reproduced periodically under ideal conditions. The past 50 years have seen the genetic diversity of the *Cannabis* genome dwindle away. Indeed, the vast majority of landraces may already be extinct, and we therefore must be careful to preserve and multiply what remains. As Watson and Clarke (1997) warned,

"Many local landrace varieties, the result of hundreds of years of selection for local use, have been lost because of *Cannabis* suppression and eradication, neglect on the part of agricultural officials and industry, anti-hemp propaganda and the general trend (until recently) to reduce industrial hemp breeding and research. Genetic materials are a living heritage and we are their custodians. We must concentrate our efforts to collect, preserve, characterize and utilize the remaining *Cannabis* genetic resources before it is too late."

As the worldwide reduction in *Cannabis* diversity continues, the importance of genetic preservation becomes more obvious. Unfortunately, no comprehensive *Cannabis* germplasm collections exist. Most of the few seed accessions are held by national gene banks that may or may not share their valuable inventories with breeders in other countries. The largest collection of hemp germplasm is maintained by the Vavilov Institute of Plant Research (VIR) in St. Petersburg, Russia. It presently numbers 563 seed accessions, including 23 possible drug accessions from Afghanistan, Kazakhstan, Syria, Turkey, and Uzbekistan, while the remaining are all hemp and feral accessions from Armenia, Bulgaria, Chile, China, Czechoslovakia, Estonia, France, Germany, Hungary, Italy, Latvia, Moldova, Poland, Portugal, Romania, Russia, Spain, Sweden, Ukraine, the United States, and former Yugoslavia (Grigorev, 2015). Since the late 1980s, political, technical, and financial difficulties in Russia have resulted in low population sizes and incomplete isolation, and consequently there has been considerable loss of genetic diversity and purity in the VIR collection (Hillig, 2004b). Many accessions may now be so similar to each other that their importance to future breeding programs could be diminished.

In 1992, the *Cannabis* germplasm collection at Wageningen University in the Netherlands contained over 156 accessions originating from 22 countries and largely sourced from other collections and research institutes (De Meijer and van Soest, 1992; Gilmore *et al.*, 2007). Nearly half of these accessions are from the former USSR and Hungary. The Institute of Natural Fibres and Medicinal Plants gene bank collections in Poland contain 139 accessions of predominantly European origin, with accessions from France, Hungary, and the Ukraine contributing 54.7% of the collection (Mankowska and Silska, 2015). The Yunnan Academy of Social Sciences collection in Yunnan province, China holds approximately 350 accessions mostly of East Asian origin (Salentijn *et al.*, 2014) and the Ecofibre Global Germplasm Collection in Australia contains additional Eurasian accessions (Welling *et al.*, 2015). However, comprehensive accession data are sorely lacking in several of these collections and this limits their value to breeders (Welling *et al.* 2016).

In addition, the subterranean Svalbard Global Seed Vault on the Norwegian island of Spitsbergen about 1300 km (810 miles) from the North Pole has a total of 43 *Cannabis* accessions that are duplicated in three other seed banks. Five of these accessions, from North Korea, Netherlands, Spain, Syria, and Turkey, may possibly be *Cannabis* drug populations; 21 others are hemp accessions from Argentina, Austria, China, Croatia, France, Georgia, Germany, Italy, Poland, Romania, Slovakia, Spain, and Sweden; and 16 accessions are of unknown origin (http://www.nordgen.org/sgsv/). The world's largest seed repository is the Millennium Seed Bank housed at the Wellcome Trust Millennium Building in West Sussex, near London, which specializes in wild plants and has only one *Cannabis* accession, which is from Slovakia (http://apps.kew.org/seedlist/SeedlistServlet).

Given the importance of *Cannabis* as a traditional as well as present-day crop plant, the biodiversity of this genus (particularly among the drug cultivars) is sorely under-represented in seed banks, especially in light of recent research interest in medical *Cannabis*. If we take into account this lack of diversity, in light of genetic impurity and low seed numbers, there really is no reliable reserve of *Cannabis* seeds. The primary goal of germplasm preservation is the conservation of the entire genome of each population. It is especially important in open-pollinated, cross-breeding plants that the population size is large enough to ensure that as many of the alleles as possible within each gene pool are reproduced in the seed. A minimum of 1000 plants for monoecious accessions, and 2000 plants for dioecious accessions, assures that 99% of the *Cannabis* alleles will be reproduced (Crossa *et al.*, 1993). Unfortunately, the seed reserves of many of the *Cannabis* seed bank accessions consist of less than 1000 viable seeds (often only 500 or less); therefore, genetic diversity is already limited by the

number of archived seeds. The secondary goal of genetic preservation is to reproduce the accessions in sufficient quantities to maintain a reserve for future reproductions and public distribution.

A common goal of *Cannabis* breeders should be establishing a more comprehensive core collection of *Cannabis* seed accessions that have been exhaustively characterized agronomically in the field, and on molecular levels, genetically and chemically, in the laboratory. Only then, can we see what diversity really is available for researchers to work with in the future. This core collection should be maintained with optimal reproduction and storage methodology, and individual accession evaluations should be made accessible to breeders (Watson and Clarke, 1997).

In the past 20 years the situation has only stagnated. According to Welling *et al.* (2016), in their fine review of the present state of *ex situ Cannabis* germplasm collections:

"Coordinated and comprehensive conservation and characterization of *ex situ Cannabis* resources holds the promise of preserving genepool diversity and enabling cultivar development. However, the legal constraints imposed by international narcotics conventions over more than 50 years have been influential in the fractionation and erosion of publicly accessible *Cannabis ex situ* genetic resources. The restrictions on legal exchange of *bona fide* research materials continues to limit the establishment of physical and centralized *ex situ* core collections."

XI. Present and future directions for *Cannabis* breeding start here!

The primary evolutionary process that is presently furthering domestication in *Cannabis* is basic Mendelian breeding; this traditional system involves selecting simply inherited traits and increasing their homozygosity through sexual recombination. Many of the primary economic traits of *Cannabis* are simply inherited (e.g., stalk height, seed size, and cannabinoid content). Cannabinoid biosynthesis is controlled by a narrow range of alleles limited to only a few loci; heritability is extremely high, which has favored successful breeding for high-THC and high-CBD sinsemilla cultivars as well as low-THC industrial hemp cultivars. High variability and strong heritability also influence both flowering and maturity times, and breeding for earlier-maturing drug varieties and later-maturing fiber varieties continues. Selection for reduced sensitivity to day length could improve the versatility of fiber hemp varieties making breeding and seed multiplication easier. Late-maturing or nonflowering fiber hemp varieties with low THC content would significantly improve biomass yield and extend the range where fiber hemp can be grown economically into sub-tropical and equatorial regions (Salentijn *et al.*, 2014). Indeed, breeders are expected to make further advances on all fronts as *Cannabis* cultivation becomes more widespread for a variety of uses.

There are many achievable goals awaiting twenty-first-century hemp breeders. Fiber hemp breeders will certainly focus on raising fiber content and yield (e.g., increasing fiber percentage, extending vegetative period), improving stalk and fiber quality for specific uses (e.g., paper pulp, composites, and fine textiles), altering cannabinoid content and composition (e.g., low THC percentage with high CBD yield), developing cultivars that are easier to process (e.g., faster retting, easier peeling, and cheaper dyeing), and increasing resistance to pests and diseases. Hemp fiber is more easily extracted and processed when the fiber bundles are high in cellulose and low in lignins and pectins that bind the fibers together within the bundles. Sequencing the genes responsible for the production of these compounds and the ways they are assembled could assist in cultivar development for the textile and paper industries (Mandolino and Carboni, 2004).

Seed hemp breeders could develop cultivars with specific protein and fatty acid compositions tailored for specific whole seed, kernel, and oil markets, as well as easily processed (e.g., harvesting, threshing, and hulling) cultivars, and should learn to control monoecy for hybrid seed production (Salentijn *et al.*, 2014).

Industrial hemp cultivation is presently restricted to temperate latitudes, but its economic range could be widely enlarged by developing cultivars adapted to lower latitudes, or by selecting for day-neutral cultivars that could be grown at any latitude. All commercial hemp cultivars are presently adapted to growing at or above 45 degrees latitude, and will yield less fiber when they are relocated as little as two degrees closer to the equator. Closer to the equator, the day length is shorter earlier in the summer, and consequently the *Cannabis* crop will flower prematurely when plants are small, which lowers fiber and seed yield. European hemp cultivars cultivated closer to the equator than within their normal latitudinal farming range are also attacked by a host of alien pests and diseases for which they have little resistance. As a result, no hemp varieties approved by the EU can be successfully grown closer to the equator than at about 40 degrees latitude. Limited traditional hemp production in other regions of the world relies mostly on unimproved local landraces (Clarke, 1995, 2007; Watson and Clarke, 1997; Clarke and Gu, 1998).

Further advances in industrial hemp breeding are also expected in the near future. Economically efficient fiber production relies on increasing the scale of production,

reducing production costs, and tailoring production for specific markets. These goals could be achieved in part by breeding for increased yield of biomass along with increased fiber yield and quality, reducing waste such as dust, and improving fiber color. Field experiments on small test plots indicate that hemp may have the potential to produce much higher stalk and fiber yields than are presently achieved. Improved yields of both fiber and seed varieties could be attained by continued selection for optimum flowering date, enhanced resistance to pests and diseases, and improved canopy architecture. Improvements developed by breeders for specific uses, such as fiber size, aspect ratio, strength, stiffness, density, surface characteristics, adhesive properties, lignification, and color, must be carried out in concert with development of optimized agronomic regimes and specific processing strategies for woven, nonwoven, and composite applications. Ideal hemp fiber varieties should have a high proportion of fine and easily extracted fibers that enhance rapid processing, as well as producing less waste. However, fiber quality and ease of retting are controlled by a complex set of genes which interact with agronomic and processing conditions that result in a variety of difficulties for fiber hemp breeders, including lower heritability of fiber traits. On the other hand, seed fatty acid and protein syntheses are controlled by fewer genes encoding for specific products; this makes selection and breeding for seed traits more straightforward (Weightman and Kindred, 2005).

One of the primary goals of EU hemp breeders over the past several decades has been the reduction of THC levels. In 2001, the EU lowered allowable THC levels to 0.2%, although previously mandated at below 0.3%. This legal reduction of allowable THC levels created problems for hemp breeders faced with maintaining cultivar productivity while further reducing already negligible psychoactive drug content. The lowering of THC levels encouraged breeders to further increase inbreeding with monoecious lines and was a major factor leading to reduced genetic variability in present-day European cultivars. Breeders are now working on "medicinal hemp" drug cultivars that are high in CBD while remaining low in THC. There is an ancient tradition of growing multipurpose *Cannabis* plants in various areas of China. However, very little had been cultivated during modern times, until Chinese farmers recently began growing hemp more intensely for fiber and seed as well as by-product CBD production. Indeed, two high-CBD industrial hemp cultivars should have been registered, with sowing seed released by the Yunnan Academy of Agricultural Sciences in 2016 (Salentijn *et al.*, 2014).

Two predominant trends of *Cannabis* breeding have appeared in recent decades. On the one hand, *Cannabis* has been modified by industrial hemp breeders who have lowered THC content and raised fiber content while developing uniform, high-yielding cultivars. On the other hand, sinsemilla breeders have also altered the genetic makeup of drug varieties effecting quantitative traits such as raising THC content, flower to leaf ratio in the inflorescences, and yield of flowers. Intense selection for potency explains in part why drug cultivars are "generally less polymorphic and heterozygous than hemp cultivars" (Weiblen *et al.*, 2015). Additional qualitative traits in drug cultivars have also been altered recently such as increasing branching, shortening internodes, and selecting for rapid maturation and early floral response to inductive photoperiods.

The following four key evolutionary events differentiate drug cultivars from fiber and seed cultivars: (1) rampant and continued blending of the modern domestic, hybrid drug gene pool; (2) exportation of "improved" drug seed; (3) gene flow of modern hybrid traits back into traditional drug populations; and (4) the establishment of unique, asexually reproduced clonal drug cultivars. Recent stages in the evolution of modern drug *Cannabis* varieties have few parallels in the simple breeding history and legal distribution of hemp.

Although industrial hemp breeders still develop fiber and seed cultivars, the major thrust of *Cannabis* breeding today is directed toward developing drug cultivars, especially those with enhanced medical efficacy. Recycling of public domain "genetics" continues, but sinsemilla breeders are increasingly reexamining original landraces and older hybrids to revitalize their breeding programs, while the quest to collect traditional landrace parents continues.

The present-day NLD/BLD hybrid gene pool, despite its recent history of incest, continues to produce novel allelic combinations. Breeders have recently developed new sinsemilla cultivars with enhanced efficacy in treating particular medical indications. The worldwide spread of BLD genes into traditional NLD regions has nearly brought an end to pure NLD landraces. In the face of this degradation of in situ conservation, breeders are developing new cultivars by growing some of the few remaining pure NLD and BLD landrace seeds. NLD landraces are valued in hybrid crosses to impart particular aromas and flavors or to enhance potency and diversify effects. Traditional NLD landrace varieties imported from India, Kashmir, Nepal, Indonesia, Korea, Southeast Asia and Mexico, as well as areas in western, central and southern Africa, have regained favor because they mature relatively early, but express fewer of the undesirable traits of BLD landraces (e.g., low flower to leaf ratio, fungal susceptibility, and unpleasant aromas). However, many of these traditional NLD landrace varieties have

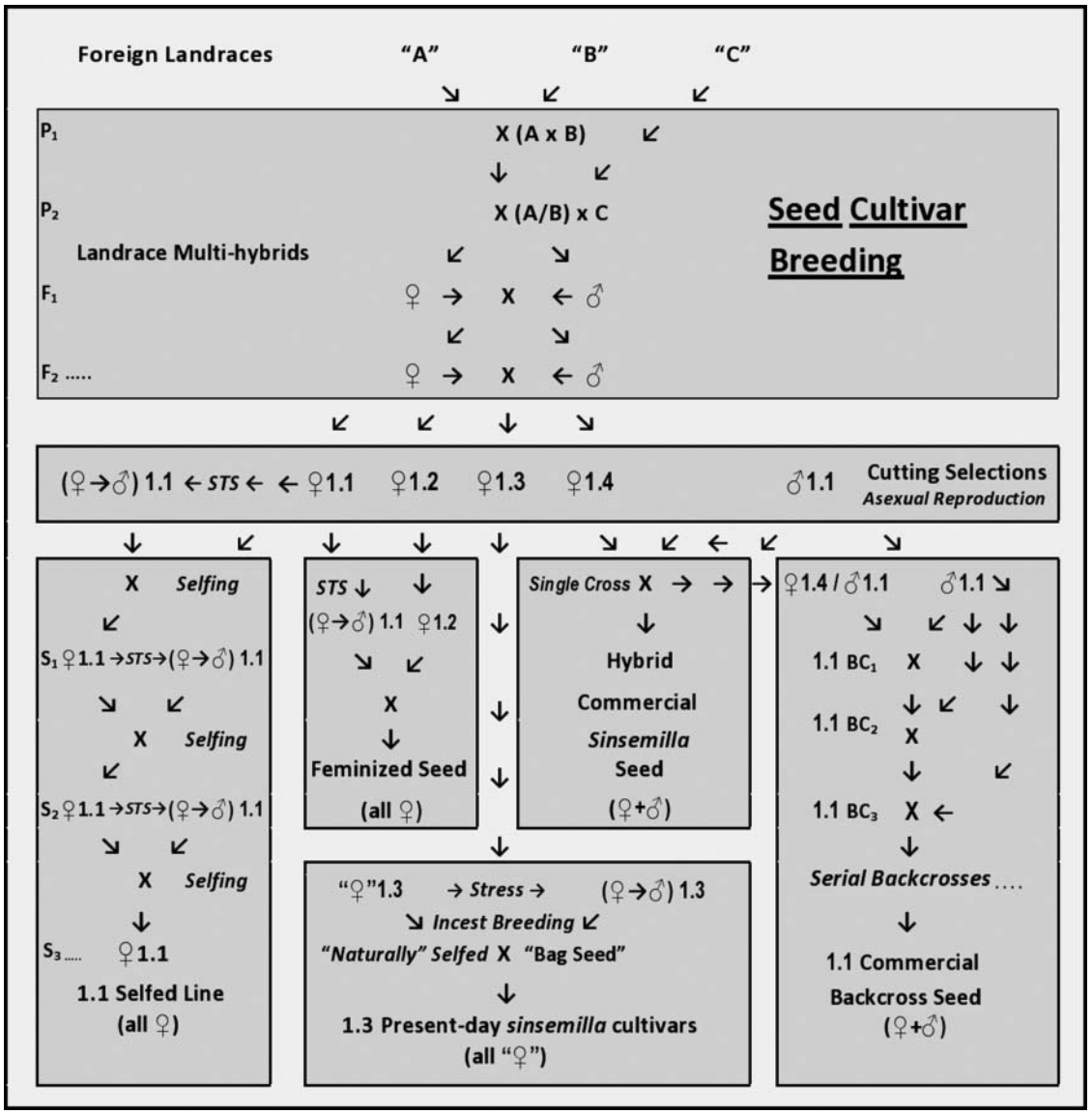

Figure 17. Cultivar development begins by combining two or more landraces through parental (P) generations to create a diverse parental population. Select female and male siblings from the parental population are then crossed for several filial (F) generations to form a synthetic multi-hybrid. The multi-hybrid seeds are subsequently grown out and female and male plants selected for breeding are asexually reproduced by rooting vegetative cuttings. At this point, five different intentional and unintentional "breeding" paths can be followed. The most common is to cross a female clone (1.4) with a male clone (2.1) to produce single-cross F_1 hybrid male and female seed. A related technique using the same parents would be to backcross (BC) a select F_1 female (1.4 × 2.1) to the same male parent (2.1), sometimes for multiple generations, to produce backcross hybrid male and female seeds. These traditional breeding techniques have been practiced for decades. The next three twenty-first-century pathways rely on female plants to produce pollen. Plant growth regulators, such as colloidal silver ions, are intentionally applied to female (XX) plants (1.1) to initiate male flowers that will produce viable female (XX) pollen. This pollen can be applied back to the same female clone (1.1) to make selfed (S) (1.1) all-female lines to increase homozygosity in breeding, or it can be crossed to another female clone (1.2) to make (F_1) all-female sowing seed. The accidental fifth path mimics the previous two, except male flowers infrequently appear spontaneously in "female" clones (1.3), release pollen unnoticed by the grower, and fertilize the same (1.3) or other clones unintentionally. This is the recent incestuous lineage of many modern-day *sinsemilla* cultivars.

become more difficult to procure in recent years. Breeders only recently learned that Afghan BLD landraces produce CBD (largely bred out of modern hybrid sinsemilla varieties) and new accessions from Central Asia are occasionally also introduced. In addition, breeders are also developing hashish varieties by selecting traits such as large and easily removed resin glands, dry rather than sticky or oily resin texture, and aromatic profiles that persist through various processing protocols. Because commercial-size marijuana shipments do not often originate in traditionally noncommercial regions, landrace seeds are usually collected in small numbers by

travelers, and are relatively rare compared to imported seeds from the major marijuana producers (Figure 17).

Sinsemilla growers prefer clones with a number of economically desirable traits: (1) high yield of dry biomass; (2) high proportion of flowers as opposed to leaves and stems; (3) preponderance of large glandular trichomes; (4) high total cannabinoid content in the flowers; (5) reproducible profile of the target cannabinoids (e.g., CBD, THC, etc.); and (6) desirable suite of aromatic terpenes—these are the major traits that breeders attempt to accentuate. Similar to sinsemilla breeding, the development of cultivars to supply pharmaceutical raw materials starts with several promising hybrid crosses, followed by serial inbreeding of select individuals from the hybrid population and/or backcrosses to a parental line. When successful, hybrid vigor is restored by making crosses between selected inbred lines (de Meijer, 2004). "Medisins" is a vegetatively reproduced "Skunk No. 1" high-THC pharmaceutical cultivar registered by Horta-Pharm in the Netherlands in 1998, and "Grace" is a high-CBD seed cultivar registered by GW Pharmaceuticals in the United Kingdom in 2004; both of these cultivars have been awarded plant breeders rights (Weightman and Kindred, 2005). Cultivars can also be developed that produce other cannabinoid compounds of medical or industrial interest, and secondary metabolite synthesis can be altered to provide enhanced protection from pests and pathogens.

Developing sterile cuttings of female varieties or seed varieties that are infertile would guarantee that female plants will be seedless even when grown near pollen sources such as hemp seed fields, and offer an additional advantage to commercial sinsemilla growers. More cultivars will be developed that produce economically valuable amounts of rare cannabinoids (e.g., cannabichromene (CBC), cannabigerol (CBG), etc.). Cultivars could also be developed that produce predominately one aromatic terpenoid compound or suites of varying terpenoids in fixed amounts. These new cultivars may prove to be of pharmaceutical interest, and competition may develop between plant breeders that will enhance the production of these rare cannabinoid compounds. In addition, the bio-engineering manipulation of cannabinoid synthases by microorganisms may lead to innovative production systems of these or other rare cannabinoids. Among the major attractions of sinsemilla cultivars are their diverse aromas and flavors. However, breeding for differing parameters may prove difficult because terpenoid synthesis pathways are controlled by a suite of genes.

In the future, legal *Cannabis* enterprises will rely on the protection of intellectual property rights. Uncommon and readily apparent biological traits could be added through breeding or genetic modification (GM, see below) to identify novel cultivars and protect the intellectual property of the breeder. These traits might include recessive morphological markers such as abnormal pigmentation (e.g., yellow stem, purple flowers, or red leaves); abnormal leaf shapes (e.g., hooked serrations or webbed leaves); or unique chemical profiles (e.g., traces of a minor unique aromatic terpenes or aldehydes) that would make the cultivar phenotypically distinct. Genetic fingerprinting of DNA sequences will also be used to identify cultivars.

Biotechnology in the form of GM has already reached *Cannabis*. Japanese researchers transferred the THC synthase gene from *Cannabis* to cultivated tobacco (*Nicotiana tabacum* L.) and induced it to convert CBG (cannabigerol, the precursor molecule to THC) into THC (Shoyama *et al.*, 2001). German researchers have engineered a yeast (*Pichia*) to make the same synthetic step and plan to insert more genes for earlier biosynthetic steps along the pathway to THC (Zirpel *et al.*, 2015). Other agronomically valuable traits may also be transferred to *Cannabis* such as enhanced pest resistance, increased yields of medically valuable compounds, tolerance of environmental extremes, and sexual sterility.

Recently, breeding lines that are focused primarily on oleic acid production have been created using gene Targeting Induced Local Lesions in Genomes (TILLING) techniques; this method allows identification of mutations in a specific gene. Vegetable oils with high oleic acid content are more stable at room temperature than those rich in essential fatty acids (EFAs), *alpha*-linolenic acid (an *omega*-3 fatty acid) and linoleic acid (an *omega*-6 fatty acid), which are both commonly found in *Cannabis* seed. Bielecka *et al.* (2014) identified a suite of alleles for "putative desaturase genes representing the four main activities required for production of polyunsaturated fatty acids in hemp seed oil," and by gene TILLING a chemically mutagenized population, they were able to select plants that lacked the desaturase genes. Four backcrosses and sibling crosses achieved homozygosity. When grown in the field, offspring of this population produced seeds with a 70% oleic acid oil content and total oil yields similar to parental lines; this "lays the foundation for the development of additional novel oil varieties in this multipurpose low input [*Cannabis*] crop" (Bielecka *et al.*, 2014).

The reaction to development and release of GM organisms has been guarded in general and will be with *Cannabis* as well (Russo, 2011). *Cannabis* presents a particularly high risk of transmitting GM genes to industrial hemp crops and weedy *Cannabis* because it is wind-pollinated, although so is GM maize that is widely

grown. The EU has installed strict regulations to prevent the accidental release of GM genes, and therefore production of GM *Cannabis* in the EU may prove impractical. However, nonfood industrial fiber and pharmaceutical *Cannabis* cultivars may not receive as much resistance from consumers and environmentalists as food crops. Genes coding for cannabinoid biosynthesis might also be transferred from *Cannabis* to organisms that are less politically sensitive than tobacco such as single-cell organisms. Transferring cannabinoid biosynthesis genes from *Cannabis* into other plants, fungi, and bacteria opens up the possibility of producing medically valuable cannabinoids in industrial fermenters and circumventing *Cannabis* growing altogether. However, *Cannabis* plants already produce extremely high yields of target compounds. Dried female sinsemilla inflorescences can yield 20% of THC or CBD, which is very high for a target compound. With the widespread, recently increasing legalization of *Cannabis* cultivation and development of high-yielding cultivars for cannabinoid extraction, genetically engineered microorganisms will face much competition for market share.

Molecular markers for suites of genes associated with biosynthesis of individual target compounds as well as desirable agronomic traits would allow mass screening of juvenile populations; this could be of great advantage to breeders (de Meijer, 2004). Sequencing cannabinoid synthase alleles would allow marker-assisted breeding for development of single cannabinoid cultivars and could be especially useful in recognizing rare cannabinoid variants (e.g., plants with increased production of cannabigerol (CBG), tetrahydrocannabivarin (THCV), or cannabichromene (CBC)) for pharmaceutical production (Mandolino and Carboni, 2004). Once the entire *Cannabis* genome is mapped and a reference genome established, researchers can begin excising and inserting sequences coding for each trait. Next-generation genome sequencing technology will allow breeders to explore the diversity of the *Cannabis* genome, measure relatedness between individuals, identify gene sequences linked to certain economic traits, screen thousands of individuals for rare mutations, and use marker-assisted selection to improve complex traits (Salentijn *et al*., 2014).

One important insight revealed by recent *Cannabis* genome projects is how inbred the drug *Cannabis* genome has become—most clones are closely related to all the others. It will be beneficial to breeders to choose parents that are less related (i.e., ones with a greater genetic distance between them) in order to encourage hybrid vigor and reduce inbreeding depression. Initial studies aimed at forensic identification of illicit cannabis concluded that the genome is highly heterozygous due to its obligate outcrossing with most alleles appearing at low frequency. With the exception of certain major alleles shared by inbred cultivars (e.g., "Fibrimon" relatives), alleles are shared across a wide spectrum of phenotypes, and consequently there is a high degree of variation even within inbred female cultivars. These are all factors that have so far limited the application of genome data for taxonomic, evolutionary, forensic, and plant improvement research (Salentijn *et al*., 2014). Only more recently have genetic differences between *Cannabis* hemp and drug populations become more clear (Van Bakel *et al*., 2011; Sawler *et al*., 2015).

XII. Summary and conclusions

Cannabis is an annual, sun-loving plant that thrives in open, nitrogen-rich environments, including rubbish piles created by humans (Anderson, 1967; Merlin, 1972; Clarke and Merlin, 2013; also see Small, 2015). *Cannabis* also has relatively large, numerous, and easily sown seeds, and therefore was preadapted for cultivation. Close associations between humans and *Cannabis* stimulated its early cultivation, and over time this eventually led to its domestication. As ancient agricultural strategies took form, humans began to select plants that provided more and better products. Some of the earliest seed selections perished and growers must have constantly collected from spontaneous populations to replace, supplement, or modify extant varieties, resulting in evolution of improved landraces. As their familiarity with *Cannabis* grew, humans became more discerning with their seed selections.

Because *Cannabis* grew well as a camp follower, later selections were increasingly made from feral populations near human settlements, rather than truly wild populations. Early semi-domesticated varieties also became naturalized and interbred with nearby feral and/or wild populations. Selection for differing economic traits continued as *Cannabis* spread well beyond its original putative range in Central Asia. Backcrosses between cultivated and truly wild populations became rare, whereas crosses between cultivated and feral populations became more common. Eventually, isolation developed between wild, cultivated, and feral populations which evolved their own phenotypes. This scenario led to the extreme variation encountered today in geographically isolated populations, and accounts for the absence of a ubiquitous, single spontaneously growing biotype throughout Eurasia.

Profound evolutionary changes can occur rapidly in annual plants such as *Cannabis*, and because it was likely abundant in its original natural environment, repeated selection was possible. Under such conditions, early humans probably did not worry about improving yields, collecting enough from nearby populations to satisfy immediate needs. Only along with the advent of *Cannabis* management and eventually cultivation did an

interest arise in increasing yields. As an intimate relationship between each plant population and the indigenous people developed, selection and controlled breeding began.

The economically valuable traits of *Cannabis* such as its strong bast fiber, edible seed, and psychoactive resin are readily noticed and appreciated, and its first use was likely very early in human history in the regions where it was first encountered. The biology and ecology of *Cannabis* were determined by when and where it evolved—temperate Central Asia during an ancient warm interglacial period being the most likely choice for the origin of *Cannabis*, a sun and warmth-loving, water and nutrient hungry, dioecious, wind-pollinated, short-day annual plant. Although this genus was undoubtedly useful to early peoples in its naturally evolved state, human selection has played the major role in its evolution as a cultivated species and allowed *Cannabis* to thrive almost everywhere it has been introduced. Selection and breeding of *Cannabis* are the means by which humans exert the most control over its evolution as a domesticated crop plant, and lie at the heart of the human–*Cannabis* relationship. Artificial selection has raised fiber content, increased seed size and yield, and both raised and lowered cannabinoid levels. Cannabinoid chemotypes are characteristic of ultimate geographical origin and usage groups; and they are correlated with movements of *Cannabis* germplasm by humans to new environments where they were affected by local cultural selection for particular products. *Cannabis* is now grown legally in many regions of the world.

Presently, few if any imported drug *Cannabis* shipments are as psychoactively potent as the best sinsemilla grown in North America and Europe. However, seeds of improved hybrid cultivars have spread to many *Cannabis* producing nations, and the potency of *Cannabis* originating from select locations is steadily increasing. Because of continuing international pressure against marijuana growers, and the highly inflated price of sinsemilla, indoor cultivation will maintain popularity worldwide in any location with a reliable electrical grid. Instructional information is readily available on the Internet, and it is easy to install and operate grow rooms in attics, bedrooms, or basements. Under these crowded circumstances, there is no tolerance for nonproductive plants, and the single most productive female clone is usually selected for all future cultivation. The use of a single clone improves grow room performance, but precludes the possibility of seed production. Breeding is no longer possible and variety improvement ceases entirely. Vegetative propagation essentially freezes evolution, and gene diffusion via cuttings has been more localized and much slower than with seeds. Presently, due to the rapid spread of indoor cultivation, growers are increasingly likely to exchange vegetative cuttings of proven clones rather than carefully bred seeds, and consequently the genetic diversity in drug *Cannabis* cultivars has decreased in the past two decades. As cannabis prohibition fades, production is rampantly proliferating in existing agricultural regions, and in the near future only the most productive and profitable clonal cultivars will be grown. Cloning will have a lasting effect on sinsemilla production and will further slow the evolution of drug *Cannabis*.

Economic competition will increase with legalization and normalization, and *Cannabis* will be further domesticated as just another crop, with high yields and ease of growing taking precedence over other traits. Although traditional smoked marijuana will always have its followers, and some connoisseur cultivars and their boutique growers will prevail as they have in the wine industry, the bulk of future production will probably be extracted for use in edible and vaporized delivery systems. If target compounds in the future are derived from a limited number of industrial drug cultivars, *Cannabis* genetic diversity will once again suffer.

Present-day clonal sinsemilla cultivars remain geographically and functionally isolated from gene exchange with their parental populations in Afghanistan or other regions of origin. However, many NLD/BLD hybrid seeds were produced in Western countries and some did interbreed with original ancestral populations. Well-meaning travelers visited many regions where NLD landraces were still growing and gave modern hybrid seeds to local farmers who crossed them with their traditional landraces, hoping for economic benefit. In the process, these traditional landraces are becoming contaminated and eventually displaced by the introduced hybrids. Original pure NLD landraces have become rare in all traditional marijuana producing countries (e.g., Jamaica, Mexico, Morocco, and Thailand). This situation makes the collection of traditional landrace varieties increasingly difficult, and consequently their preservation and reproduction are more significant issues than ever before. It is important to collect landrace building blocks on which future breeders will depend for creating improved cultivars; for the sake of genetic diversity, we must escape from our reliance on clones and their inherent evolutionary stagnancy. Save and sow your seeds!

References

Agrios, G. N. 2005. *Plant Diseases Caused by Fungi in Plant Pathology*. 5th ed., pp. 137, 268, 467–468. Elsevier Academic, Amsterdam.

Anderson, E. 1967. *Plants, Man, and Life*. (Original edition 1952). University of California Press, Berkeley.

Berenji, J., Sikora, V., Fournier, G., and Beherec, O. 2013. Genetics and selection of hemp. In: *Hemp: Industrial Production and Uses*, pp. 48–71. Bouloc, P., Allegret, S., Arnaud, L., Eds., CABI, Wallingford, UK.

Bielecka, M., Kaminski, F., Adams, I., Poulson, H., Sloan, R., Li, Y., Larson, T. R., Winzer, T., and Graham, I. A. 2014. Targeted mutation of Δ^{12} and Δ^{15} desaturase genes in hemp produce major alterations in seed fatty acid composition including a high oleic hemp oil. *Plant Biotechnol. J.* **12**(5): 613–623.

Bócsa, I. 1994. Professor Dr. Ivan Bócsa, the breeder of Kompolti hemp (interview by the JIHA). *J. Int. Hemp Assoc.* **1** (2): 61–62.

Boyce, S. S. 1900. *Hemp (Cannabis sativa)*. Orange Judd Company, New York.

Clarke, R. C. 1977. *The Botany and Ecology of Cannabis*. Pods Press, Ben Lomond.

Clarke, R. C. 1981. *Marijuana Botany: An Advanced Study: The Propagation and Breeding of Distinctive Cannabis*. And/Or Press, Berkeley.

Clarke, R. C. 1998. *Hashish!* Red Eye Press, Los Angeles.

Clarke, R. C. 2001. Sinsemilla heritage: what's in a name? In: *The Cannabible*, pp. 1–24. King, J., Ed., Ten Speed Press, Berkeley.

Clarke, R. C. 1995. Hemp (*Cannabis sativa* L.) cultivation in the Tai'an District of Shandong Province, Peoples Republic of China. *J. Int. Hemp Assoc.* **2**(2): 57, 60–65.

Clarke, R. C. 2007. Traditional *Cannabis* cultivation in Darchula district, Nepal—seed, resin and textiles. *J. Ind. Hemp* **12** (2): 19–42.

Clarke, R. C. and Gu, W. 1998. Survey of hemp (*Cannabis sativa* L.) use by the Hmong (Miao) of the China/Vietnam border region. *J. Int. Hemp Assoc.* **5**(1): 1, 4–9.

Clarke, R.C. and Merlin, M. D. 2013. *Cannabis: Evolution and Ethnobotany*. University of California Press, Los Angeles and Berkeley.

Clarke, R. C. and D. P. Watson. 2007. Cannabis and natural Cannabis medicines. In: *Marijuana and the Cannabinoids*, pp. 1–17. ElSohly, M. A., Ed., Humana Press, Springer, New York.

Crossa, J., Hernandez, C. M., Bretting, P., Eberhart, S.A., and Taba, S. 1993. Statistical genetic considerations for maintaining germplasm collections. *Theor. Appl. Genet.* **86**: 673–678.

Datwyler, S. L., and Weiblen, G. D. 2006. Genetic variation in and (*Cannabis sativa* L.) according to amplified fragment length polymorphisms. *J. Forensic Sci.* **51**: 371–375.

Faeti, V., Mandolino, G., and Ranalli, P. 1996. Genetic diversity of *Cannabis sativa* germplasm based on RAPD markers. *Plant Breed.* **115**: 367–370.

Fleming, M. P. and Clarke, R. C. 1998. Physical evidence for the antiquity of *Cannabis sativa* L. *J. Int. Hemp Assoc.* **5**(2): 80–93.

Forapani, S., Carboni, A., Pioletti, C., Moliterni, V. M. C., Ranalli, P., and Mandolino, G. 2001. Comparison of varieties using random amplified polymorphic DNA markers. *Crop Sci.* **41**: 1682–1689.

Freinkel, S. 2009. *American Chestnut: The Life, Death, and Rebirth of a Perfect Tree*. University of California Press, Berkeley.

Gilmore, S., Peakall, R., and Robertson, J. 2007. Organelle DNA haplotypes reflect crop-use characteristics and geographic origins of *Cannabis sativa*. *Forensic Sci. Int.* **172**: 179–190. doi: 10.1016/j.forsciint.2006.10.025

Grigorev, S. V. 2015. *Personal communication. Dr. Grigorev is the curator of the Cannabis germplasm collection at the VIR in St. Petersburg*, Russia.

Harlan, J. R. 1965. The possible role of weed races in the evolution of cultivated plants. *Euphytica* **14**: 173–176.

Heiser, C. B. 1973. Introgression re-examined. *Bot. Rev.* **39**(4): 347–366.

Hennink, S. 1994. Optimization of breeding for agronomic traits in fiber hemp (*Cannabis sativa* L.) by study of parent-offspring relationships. *Euphytica* **78**: 69–76.

Hillig, K. W. 2004a. A chemotaxonomic analysis of terpenoid variation in Cannabis. *Biochem. Syst. Ecol.* **32**: 875–891.

Hillig, K. W. 2004b. A multivariate analysis of allozyme variation in 93 *Cannabis* accessions from the VIR Germplasm collection. *J. Ind. Hemp* **9**(2): 5–22.

Hillig, K. W. 2005a. *A Systematic Investigation of Cannabis*. PhD Dissertation. Indiana University, Bloomington.

Hillig, K. W. 2005b. Genetic evidence for speciation in *Cannabis* (Cannabaceae). *Genet. Res. Crop Evol.* **52**(2): 161–180.

Hillig, K. W. and Mahlberg, P. G. 2004. A systematic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). *Am. J. Bot.* **91**: 966–975.

Iltis, H. H. 1983. From teosinte to maize: the catastrophic sexual transmutation. *Science* **22**: 886–894.

Kelly, J. 2013. *The Graves are Walking: The Great Famine and the Saga of the Irish People*. Henry Holt and Company, New York.

Kojoma, M., Seki, H., Yoshida, S., and Muranaka, T. 2006. DNA polymorphisms in the tetrahydrocannabinolic acid (THCA) synthase gene in "drug-type" and "fibertype." *Cannabis sativa* L. *Forensic Sci. Int.* **159**: 132–140.

Lynch, R. C., Vergara, D., Tittes, S., White, K., Schwartz, C. J., Gibbs, M. J., Ruthenburg, T. C., deCesare, K., Land, D. P., and Kane, N. C. 2015. Genomic and chemical diversity in *Cannabis*. bioRxiv preprint posted online Dec. 13, 2015. doi: http://dx.doi.org/10.1101/034314

Mandolino, G. and Carboni, A. 2004. Potential of marker-assisted selection in hemp genetic improvement. *Euphytica* **140**: 107–120.

Mankowska, G., and Silska, G. 2015. Genetic resources of *Cannabis sativa* L. in the collection of the gene bank at INF&MP in Poznan. *J. Nat. Fibers* **12**: 332–340. doi: 10.1080/15440478.2014.928246

McPartland, J. M. and Guy, G.W. 2004. The evolution of *Cannabis* and coevolution with the cannabinoid receptor – a hypothesis. In: *The Medicinal Uses of Cannabis and Cannabinoids*, pp. 71–101. Guy, G. W., Whittle, G. W., Robson, P. J., Eds., Pharmaceutical Press, London.

McPartland, J. M. and Guy, G.W. 2010. THC synthase in Cannabis has undergone accelerated evolution and positive selection pressure. *Proceedings of the 20th Annual Symposium on the Cannabinoids. International Cannabinoid Research Society*, p. 43. Research Triangle Park, North Carolina.

McPartland, J. M., Clarke, R. C., and Watson, D. P. 2000. *Hemp Diseases and Pests: Management and Biological Control*. CABI, Wallingford, England.

de Meijer, E. P. M., van der Kamp, H. J., and van Eeuwijk, F. A. 1992. Characterisation of *Cannabis* accessions with regard to cannabinoid content in relation to other plant characters. *Euphytica* **62**: 187–200.

de Meijer, E. P. M. and van Soest, L. J. M. 1992. The CPRO *Cannabis* germplasm collection. *Euphytica* **62**: 201–211. doi: 10.1007/BF00041754

de Meijer, E. P. M. 1995. Fibre hemp cultivars: a survey of origin, ancestry, availability and brief agronomic characteristics. *J. Int. Hemp Assoc.* **2**(2): 66–73.

de Meijer, E. P. M., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V. M. C., Ranalli, P., and Mandolino, G. 2003. The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics* **163**: 335–346.

de Meijer, E. P. M. 2004. The breeding of *Cannabis* cultivars for pharmaceutical end uses. In: *The Medicinal Uses of Cannabis and Cannabinoids*, pp. 55–69. Guy, G. W., Whittle, B. A., Robson, P. J., Eds., Pharmaceutical Press, London and Chicago.

de Meijer, E. P. M. 2014. The chemical phenotypes (chemotypes) of *Cannabis*. In: *Handbook of Cannabis*, pp. 89–110. Pertwee, R., Ed., Oxford University Press, Oxford.

Merlin, M. D. 1972. *Man and Marijuana: Some Aspects of Their Ancient Relationship*. Fairleigh Dickinson University Press, Rutherford, NJ.

Millennium Seed Bank. (http://apps.kew.org/seedlist/SeedlistServlet) (last accessed 16-02-01).

Mölleken, H. and Theimer, R. R. 1997. Survey of minor fatty acids in *Cannabis sativa* L. fruits of various origins. *J. Int. Hemp Assoc.* **4**(1): 13–17.

Myles, S., Boyko, A. R., Owens, C. L., Brown, P. J., Grassi, F., Aradhya, M. K., Prins, B., Reynolds, A., Chia, J., Ware, D., Bustamante, C. D., and Buckler, E.S. 2010. Genetic structure and domestication history of the grape. *PNAS Online J.* 6. www.pnas.org/cgi/doi/10.1073/pnas.1009363108

Ordish, G. 1987. *The Great Wine Blight*. Sidgwick & Jackson Ltd., London.

Ordonez, N., Seidl, M. F., Waalwijk, C., Drenth, A., Kilian, A., Thomma, B. P. H. J., Poloetz, R. C., and Kema, G. H. J. 2015. Worse comes to worst: bananas and panama disease – when plant and pathogen clones meet. *PLoS Pathog.* **11** (11): e1005197. doi: 10.1371/journal.ppat.1005197

Pacifico, D., Miselli, F., Micheler, M., Carboni, A., Ranalli, P., and G. Mandolino, G. 2006. Genetics and marker-assisted selection of the chemotype in *Cannabis sativa* L. *Mol. Breed.* **17**: 257–268.

Pollan, M. 2001. *The Botany of Desire*. Random House, New York.

Posselt, U. K. 2010. Breeding methods in cross-pollinated species. In: *Fodder Crops and Amenity Grasses. Handbook of Plant Breeding*. Vol. 5, pp. 39–87. Boller, B., Ed., Springer Science and Business Media, LLC, New York.

Promusa. (http://www.promusa.org/Tropical+race+4+-+TR4).

Russo, E. B. 2006. Cannabis treatments in obstetrics and gynecology: a historical review. In: *Handbook of Cannabis Therapeutics: From Bench to Bedside*, pp. 5–34. Russo, E., Grotenhermen, F., Eds., Haworth Press, Binghamton, New York.

Russo, E. B. 2011. *Cannabis Genome Uncloaked: Commentary on the Scientific Implications*. International Cannabinoid Research Society. Retrieved from http://www.cannabinoidsociety.org/content/Cannabis_Genome_Uncloaked.pdf.

Russo, E. B., Jiang, H., Li, X., Sutton, A., Carboni, A., Del Bianco, F., Mandolino, G., Potter, D. J., Zhao, Y., Bera, S., Zhang, Y., Lu, E., Ferguson, D. K., Hueber, F., Zhao, L., Liu, C., Wang, Y., and Li, C. 2008. Phytochemical and genetic analyses of ancient cannabis from Central Asia. *J. Exp. Bot.* **59**(15): 4171–4182.

Salentijn, E. M. J., Zhang, Q., Amaducci, S., Yang, M., and Trindade, M. 2014. New developments in fiber hemp (*Cannabis sativa* L.) breeding. *Ind. Crops Prod.* **68**: 32–41.

Sawler, J., Stout, J. M., Gardner, K. M., Hudson, D., Vidmar, J., Butler, L., Page, J. E., and Myles, S. 2015. The genetic structure of marijuana and hemp. *PLoS ONE* **10**(8): eO133292. doi: 10.1371/journal.pone.0133292

Serebriakova, T. I. 1940. Fiber plants. In: *Flora of Cultivated Plants*. Vol. 5, Part 1. Wulff, E. V., Ed., State Printing Office, Moscow and Leningrad [in Russian].

Shoyama, Y., Taura, F., and Morimoto, S. 2001. Expression of tetrahydrocannabinolic acid synthase in tobacco. *Proceedings, 2001 Symposium on the Cannabinoids*. International Cannabinoid Research Society, Burlington, VT.

Sinskaja, E. N. 1925. Field crops of the Altai. *Bull. Appl. Bot. Plant Breed.* **14**(1): 367–370.

Small, E. 2015. Evolution and classification of *Cannabis sativa* (marijuana, hemp) in relation to human utilization. *Bot. Rev.* **81**(3): 189–294.

Small, E. and Beckstead, H. D. 1973a. Cannabinoid phenotypes in *Cannabis sativa*. *Nature* **245**: 147–148.

Small, E. and Beckstead, H. D. 1973b. Common cannabinoid phenotypes in 350 stocks of *Cannabis*. *Lloydia* **36**(2): 144–165.

Small, E., Beckstead, H. D., and Chan, A. 1975. The evolution of cannabinoid phenotypes in *Cannabis*. *Econ. Bot.* **29**(3): 219–232.

Small, E. and Cronquist, A. 1976. A practical and natural taxonomy for *Cannabis*. *Taxon* **25**(4): 405–435.

Small, E. and Marcus, D. 2002. Hemp: a new crop with new uses for North America. In: *Trends in New Crops and New Uses*, pp. 284–326. Janick, J., Whipkey, A., Eds., ASHS Press, Alexandria, VA.

Small, E. and Marcus, D. 2003. Tetrahydrocannabinol levels in hemp (*Cannabis sativa*) germplasm resources. *Econ. Bot.* **57**: 545–558.

Small, E. and Naraine, S.G.U. 2015. Size matters: evolution of large drug-secreting resin glands in elite pharmaceutical strains of *Cannabis sativa* (marijuana). *Genet. Resour. Crop Evol.* doi: 10.1007/s10722-015-0254-2

Svalbard Global Seed Vault. http://www.nordgen.org/sgsv/ (last accessed 16-02-01).

van Bakel, H., Stout, J. M., Cote, A. G., Tallon, C. M., Sharpe, A. G., Hughes, T. R., and Page, J. E. 2011. The draft genome and transcriptome of *Cannabis sativa*. *Genome Biol.* **12**: R102.

Vavilov, N. I. 1926. Tzentry proiskhozhdeniya kulturnykh rastenii. (The centers of origin of cultivated plants). *Bull. Appl. Bot. Genet. Plant Breed.* **16**(2): 1–248 [in Russian and English].

Vavilov, N. I. 1931. Rol Tzentralnoi Azii v proiskhozhdenii kulturnykh rastenii (The role of Central Asia in the origin of cultivated plants). *Bull. Appl. Bot. Genet. Plant Breed.* **26** (3): 3–44 [in Russian and English].

Watson, D. P. and Clarke, R. C. 1997. The genetic future of hemp. In: *Nova Institute, Bioresource Hemp Symposium Proceedings*, pp. 122–127, (Frankfurt am Main, Germany, Feb. 27–March 2, 1997), Hürth, Germany.

Weiblen, G. D., Wenger, J. P., Craft, K. J., ElSohly, M. A., Mehmedic, Z., Treiber, E. L., and Marks, M. D. 2015. Gene

duplication and divergence affecting drug content in *Cannabis sativa*. *New Phytol.* doi: 10.1111/nph.13562

Weightman, R. and Kindred, D. 2005. *Final Report for the Department for Environment Food and Rural affairs Review and Analysis of Breeding and Regulation of Hemp and Flax Varieties Available for Growing in the UK*. ADAS UK Ltd, Wolverhampton, England.

Welling, M. T., Liu, L., Shapter, T., Raymond, C. A. and King, G. J. 2015. Characterisation of cannabinoid composition in a diverse *Cannabis sativa* L. germplasm collection. *Euphytica* **208**: 463–475. doi: 10.1007/s10681-015-1585-y

Welling, M. T., Rose, T. J., Liu, L., Stanger, R. and King, G. J. 2016. A belated green revolution for *Cannabis*: virtual genetic resources to fast-track cultivar development. *Front. Plant Sci.* **7**, article 1113: 1–17.

Wikipedia. https://en.wikipedia.org/wiki/Southern_corn_leaf_blight.

Zirpel, B., Stehle, F. and Kayser, O. 2015. Production of Δ^9-tetrahydrocannabinolic acid from cannabigerolic acid by whole cells of *Pichia* (*Komagataella*) *pastoris* expressing Δ^9-tetrahydrocannabinolic acid synthase from *Cannabis sativa* L. *Biotechnol. Lett.* **37**: 1869–1875. doi: 10.1007/s10529-015-1853-x