



## Isozyme Studies of Genetic Variability

M. Thompson Conkle, Institute of Forest Genetics, Pacific Southwest Research Station, USDA Forest Service

### Abstract

Little is known about genetic variation and geographic patterns of diversity of *Acacia koa*. We analyzed diversity using laboratory studies of enzymes. Wide samples of koa possess significant variation in six enzyme genes; several genes have alleles in intermediate frequencies. The variable genes have average expected heterozygosity of 0.41; this indicates that koa has significant levels of genetic diversity to adapt to varied natural environments, and it provides evidence that tree improvement can operate on a rich genetic base.

Samples from trees on Kaua'i, O'ahu, and Maui appear closely related; all share about the same profiles for the variable enzyme genes. It is noteworthy that two populations on the Island of Hawai'i, one above Kailua and the other in Hawai'i Volcanoes National Park, have enzyme profiles that are similar to one another, but both differ substantially from the profiles of populations on Kaua'i, O'ahu, and Maui. This island-to-island differentiation indicates that it is important for foresters to plant with seedlings from local seed sources until more is known about the adaptations and growth of koa geographic races.

The introduced and naturalized *Passiflora* vine (banana poka) has potential to over-top and smother forests; it is adapted to elevations and conditions characteristic of native koa forests. Banana poka seed samples from Kaua'i, O'ahu, Maui, and the Kailua and Hawai'i Volcanoes National Park areas on Hawai'i all were found to lack genetic variation: enzyme patterns were identical throughout Hawai'i, there was no evidence of allelic variation for 14 genes, there was no evidence of genetic diversity, and the observed heterozygosity was zero.

The Hawaiian banana poka enzyme gene profile exactly matches the profile of *Passiflora tripartita* var. *tripartita* from Ecuador, Colombia, and Venezuela. The latter species, a common domesticated juice plant of the high Andes, is also genetically uniform and homozygous, with a single allele type at each enzyme gene. The other Andean candidate progenitor species, *Passiflora*

*tripartita* var. *mollissima*, has distinctly different enzyme profiles. Researchers can collect *Passiflora* biological control organisms in South America for tests on *Passiflora tripartita* var. *tripartita* with knowledge that significant genetic differences exist between the two Andean species, and with knowledge that var. *tripartita* and banana poka have identical and homozygous genetic constitutions.

### Isozyme studies of koa genetic variability

Little is known about the relative amounts of genetic variation of the geographic patterns of genetic diversity of *Acacia koa*, Hawai'i's signature wood and major forest tree species. While some workers collected seeds, planted common garden trials, and evaluated growth adaptations, we analyzed genes using laboratory studies of enzymes. Enzyme genes disclose levels of diversity and help to reveal geographic races.

Fresh seeds were collected from the Waimea Canyon, Koke'e, Alaka'i forest of Kaua'i (60 trees in the sample); Wai'anae Range (24), and also northern [Pupukea (44)], and southern Honolulu (32) forests of the Ko'olau Range, O'ahu; forest near the Hosmer Grove, Haleakala, East Maui (6); and forests on the island of Hawai'i: Hualalei above Kailua-Kona (18), and Mauna Loa Strip Road, Hawai'i Volcanoes National Park (28).

Our laboratory analyzed enzyme genes from the embryos of germinating seeds. Koa is a tetraploid, 4N plant that presents numerous isozyme band patterns. Initial analyses of seeds from individual pods, each being a full-sib family, helped to characterize the gel band patterns from six polymorphic genes (6PG2, IDH, MDH2, PGI2, GOT3, GDH). Our species analysis used one seed per tree to avoid seed parent bias and to provide wide evaluation of diversity within the respective forests. Koa population samples possess relatively large numbers of alleles per gene, from three alleles for IDH, up to seven for MDH2. Several of the genes have alleles in intermediate frequencies. Overall, the variable genes have average expected heterozygosity of 0.41.



This is a high value compared with many other organisms. It suggests that koa has significant levels of genetic diversity to adapt to varied environments. It provides evidence that tree improvement can operate on a rich genetic base.

Samples from Kaua'i, O'ahu, and Maui appear closely related; all share about the same allele frequency profiles. Figure 1 presents a diagram of genetic distances showing the relationships among populations. It is noteworthy that the two sample populations from the island of Hawai'i are similar to one another, but both differ substantially from the genetic profiles of koa from Kaua'i, O'ahu, and Maui. Big Island populations have intermediate frequencies for two alleles of 6PG2 and one allele of IDH that are absent from Kaua'i, O'ahu, and Maui samples. One allele of 6PG2 and one of IDH are present in samples from Kaua'i, O'ahu, and Maui, but absent from Hawai'i. Island-to-island differentiation indicates that it is important for foresters to plant with seedlings from local seed sources until more is known about the adaptations and growth of koa geographic races.

#### **Isozyme studies of banana poka genetic variability**

The introduced and naturalized *Passiflora* vine, banana poka, has potential to over-top and smother forests; it is adapted to elevations and conditions of native koa forests. To develop opportunities for biological control of banana poka, we assessed its genetic diversity and compared its gene profile to profiles of the most likely progenitor species.

Field collections of banana poka were made in four major outbreaks in Hawai'i by harvesting individual mature pods from numerous, separate vines. Isozyme analyses of 15 enzyme genes in seed samples from Kaua'i, Maui, and the Kailua and Hawai'i Volcanoes National Park areas of Hawai'i lacked genetic variation; enzyme patterns of all samples were identical throughout Hawai'i; each gene had one allele type; there was no evidence of genetic diversity; the observed heterozygosity was zero.

Finding genetic uniformity within Hawai'i is highly significant, because it predicts that banana poka has very little opportunity to evolve. It lacks the capacity to modify gene frequencies as a means to adapt to new challenges. The plant's environmental tolerances are set the basic adaptations of its monotypic genotype. Although it possesses poisons that effectively protect it

from many predators, any organisms that overcome its defenses will have access to a uniform host. Biological control researchers consider this an ideal situation, because their control agents can be tailored for target-specific success against a unique genotype.

It is possible that insects and diseases in Hawaiian forests may eventually adapt to banana poka. But researchers can hasten control of exotic weeds by importing their native associates to weaken them, feed on them, and reduce their reproductive capacity. What then is the parent progenitor of Hawai'i's banana poka?

While there are approximately 400 species of *Passiflora*, Hawaiian banana poka only resembles a few South American varieties, those cultivated for juice and grown extensively in gardens of small villages through the Northern Andes. Entomologist Rex Friesen, when conducting biological control research at Hilo and Volcano, determined from first-hand experience in Ecuador, Colombia, and Venezuela that two varieties closely match the morphological features of banana poka. Dr. Friesen, with assistance from South American colleagues, made extensive seed collections from the primary, candidate progenitor species: *Passiflora tripartita* var. *tripartita*, and *P. tripartita* var. *mollissima*. The collections were in villages at elevations from 2075 meters (6800 feet) up to 3100 m (10,200 feet). "Fuzzy, felt-like" describes the underside of leaves of variety *mollissima*; it is the main characteristic distinguishing it from smooth leaves of variety *tripartita* and banana poka. We hasten to note that the taxonomic nomenclature of these varieties is unsettled; various species and varietal names are in local use.

Seeds from South America were analyzed in starch gels alongside seeds from banana poka to compare enzyme gene profiles. The Hawaiian banana poka enzyme gene profiles exactly match the profiles of *Passiflora tripartita* var. *tripartita* from Ecuador, Colombia, and Venezuela. Surprisingly, our isozyme analyses of 15 genes, based on extensive collections and numerous vine samples, indicate that variety *tripartita* is also genetically uniform and homozygous and, with only a few rare exceptions, consists of a single allele type at each enzyme gene. It is now clear that banana poka's homozygosity traces to a cultivated variety that is homozygous. The other Andean candidate progenitor species, *P. tripartita* var. *mollissima*, has distinctly different enzyme profiles: it is highly variable with allelic variation (5 alleles were present in GOT2) in 12 of a total of 15



enzyme genes; observed heterozygosity reaches a high of 0.15 for Venezuelan samples (expected heterozygosities are substantially higher—0.35—indicating a breeding system with substantial inbreeding).

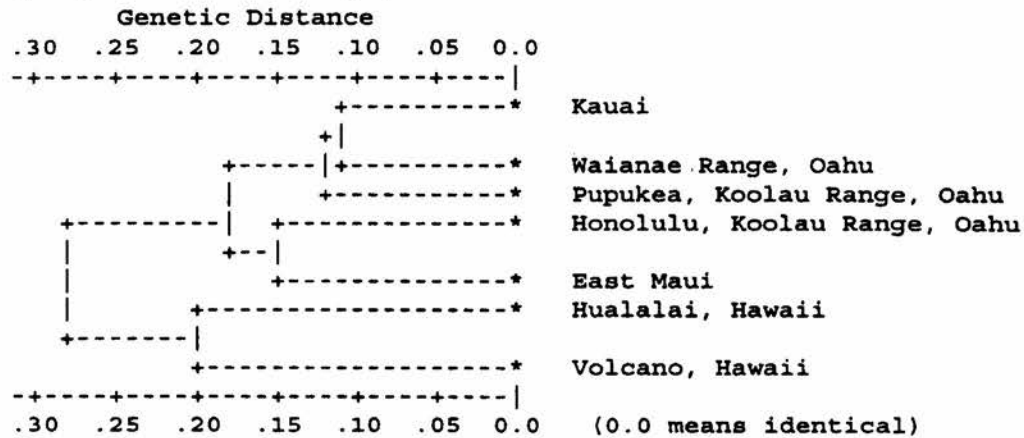
Genetic similarity relationships among banana poka and the two Andean varieties are shown in Figure 2. The two Andean species differ significantly with alternate alleles at 7 of the 15 loci.

Researchers collecting *Passiflora* biological control organisms in South America for tests on Hawaiian banana poka should concentrate their efforts on *Passiflora tripartita* var. *tripartita*. This can be done with knowledge that significant genetic differences exist between the two Andean species and with the knowledge that variety *tripartita* and banana poka have identical and homozygous genetic constitutions.

**Acknowledgments**

Funds for this research were provided by a Pacific Southwest Research Station Internal Competitive Grant, 1995. Hawaiian seed collections were accomplished with the kind permission and assistance of Marsha Erickson, Kekaha, Kaua'i; Edwin Petteys, Lihue, Kaua'i; Carl Masaki and Victor Tanimoto, Honolulu; Robert Hobdy, Wailuku, Maui; and Charles Wakida, Hilo, Hawai'i. The South American collections of *Passiflora* were made or arranged by Dr. Rex Freisen, while a Research Entomologist, Volcano, Hawai'i, Institute of Pacific Island Forestry, Forest Service, USDA. We warmly appreciate seed collection cooperation with Charlotte Causton, Venezuela, and Jaime Jaramillo Ledo, Ecuador. Seed preparation, laboratory analyses, identification of genotypes, and the analysis of genetic frequencies were accomplished by our Genetic Research Unit's highly experienced senior technician, Paul Hodgskiss, Albany, California.

**Figure 1. Pair-group clusters of Hawai'i populations of koa.**



**Figure 2. Pair-group clusters of *Passiflora tripartita* var. *mollissima*, Hawaiian banana poka, and *P. tripartita* var. *tripartita*.**

