## GENETIC DIFFERENTIATION OF HAWAIIAN BIDENS

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## Abstract

Adaptive radiation is the evolutionary divergence of a group of organisms from a common ancestor to exploit different ecological niches. Bidens has undergone extensive adaptive radiation on the Hawaiian Islands. The 19 Hawaian species exhibit much more morphological and ecological differentiation than the continental members of the genus. However, the Hawaiian taxa are chromosomally homogeneous and retain the capacity to interbreed in all possible combinations. Thus the morphological and ecological differentiation in Hawaiian Bidens has been attained without the existence of reproductive isolating mechanisms. Although some hybrid populations are known, hybridization usually does not occur in nature because the species are found in different habitats. Preliminary genetic studies have suggested that some of the morphological differences between taxa may be controlled by very few genes. Genetic differentiation may therefore not extend to other parts of the genome, such as to structural genes for enzymes. Most plant groups that have been studied electrophoretically show a correlation between morphological differentiation and genetic differentiation, but opposing predictions can be made about the extent of divergence at isozyme loci in Hawaiian Bidens. The morphological and ecological data suggest that the taxa are highly differentiated genetically, but the chromosomal similarity of species, the genetic studies of morphological characters and the absence of genetically controlled isolating mechanisms suggest that genetic differences among the taxa may
be limited to only a small portion of the genome and may not include isozyme loci.

Populations of the Hawaiian taxa of Bidens were compared at 23 loci controlling 9 enzyme systems. In general, populations are more polymorphic than populations of most other plant species that have been studied electrophoretically. Little genetic differentiation has occurred among taxa in spite of the high levels of genetic variability, however. Genetic identities calculated for pairs of populations show that populations of the same taxon are genetically more similar than populations belonging to different taxa, but all values are high. The genetic differentiation that has occurred among the taxa of Hawaiian Bidens is comparable to the genetic differences among populations of continental species. Moreover, there is no correlation between the isozyme data and morphological data. No groups of taxa are evident in the genetic data although morphological groups exist. Genetic differentiation at isozyme loci has not occurred at the same rate as the acquisition of adaptive morphological and ecological characters in Hawaiian Bidens. Adaptive radiation therefore does not require genetic change throughout the genome and may be limited to the genes controlling morphological and ecological characters.

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## I. INTRODUCTION

### 1.1 Adaptive Radiation And The Hawaiian Islands

Adaptive radiation is the evolution from a common ancestor of many species adapted to a variety of ecological niches. The evolutionary divergence of a group of organisms to exploit different environments is a significant evolutionary event, and occurred within the angiosperms in the Cretaceous, and within mammals in the Paleogene. The endemic mammals of South America and the marsupials of Australia are also examples of this phenomenon.

Evidence for adaptive radiation on a smaller scale can be seen on oceanic islands. Oceanic islands usually have unbalanced or disharmonic biotas in which genera and larger taxonomic categories are under-represented relative to continental biotas (Carlquist, 1974). The number of species in each genus, however, is often relatively large, and a high proportion of species may be endemic. This is illustrated by the many species of Darwin's finches in the Galapagos Islands (Lack, 1947) and of honeycreepers in the Hawaiian Islands (Carlquist, 1970) in conjunction with the small number of genera of land birds found on these two groups of islands. Most oceanic islands are created through volcanic activity and have never been connected to continental areas by land bridges. It is not surprising, therefore, that the genera found on them are
few in number and are usually groups with adaptations for longdistance dispersal and establishment (Carlquist, 1974). Once populations of early colonizers have been established, opportunities for speciation may be greater than on older mainland areas because of the availability of habitats not yet occupied by other species. Rapid cladogenetic evolution within a small number of genera would occur initially. As colonization by different groups of organisms continues, new ecological opportunities decrease and patterns of speciation may resemble those found on continents.

The Hawaiian Islands are geologically young and are particularly rich in habitats. The whole archipelago is 3500 kilometres long, but the major islands (Figure 1) are clustered at the southeastern end. All the islands have been formed by the movement of the Pacific plate over a fixed hot spot in the earth's mantle from which magma rises periodically to form shield volcanoes (Dalrymple et al., 1973). As the plate moves in a northwesterly direction, new islands are continually being formed and older ones are eroding to sea level and below. Although the chain itself is tens of millions of years old, the major islands range in age from 5.6 to 3.8 million years for Kauai to less than 1.0 million years for Hawaii (Stearns, 1966). Volcanic activity still occurs on Hawaii, the youngest and highest island.

A variety of habitats is available, ranging in temperature from below freezing to more than $30^{\circ} \mathrm{C}$, in humidity from nearly 0 to $100 \%$, and in median annual rainfall from over 11.8 m to less


Figure 1. Map of the major (Windward) Hawaiian Islands.
than $0.25 m$ (Carlquist, 1970; Taliaferro, 1959). The archipelago is rich in examples of adaptive radiation; in addition to the honeycreepers there are the 650-700 endemic species of Drosophila that comprise about one-third of the entire genus (Johnson et al., 1975), and many examples in flowering plants, including Lipochaeta (Gardner, 1976), Bidens, Cyrtandra, Euphorbia, Palea, Scaevola, seven genera of lobelioids (Carlquist, 1970), and three genera of the silversword alliance (Carr and Kyhos, 1981).
1.2 Hawaiian Bidens

Bidens is a worldwide genus with centres of diversity in Africa and the New World. Each of these areas has about 100 species of the total of approximately 280 in the genus (Sherff, 1937). Eurasia and the Caribbean have only about 5 species each but Polynesia has 66; a large number for such a small portion of the world's land area. Although the treatment of Sherff is in need of revision, the number of species he named probably reflects the amount of morphological and ecological diversity found in a given region. Forty-three of his Polynesian species are Hawaiian and the other 23 are found in southeastern Polynesia. They comprise 2 of the 14 sections in the genus (one consisting solely of $B$. cosmoides on Kauai) and their closest affinities are with American species. Because the southeast Polynesian species share similarities with Hawaiian species but
exhibit much less variability than is found in the Hawaiian Islands (Gillett, 1972b, 1973), it is likely that they are derived from Hawaiian taxa and that the original colonization of Polynesia from the Americas occurred in Hawaii. Similarities shared by the Polynesian species and the rarity of dispersal events to islands so remote from any continent (evidence of which is their disharmonic biotas) suggest that there was perhaps only one introduction of the genus to the islands (Ganders and Nagata, in prep.) although Fosberg (1948) and Gillett (1975) believed there were two.

The Hawaiian taxa are extremely variable morphologically. Although only a few characters, such as the helicoid achene, are unique to the islands (Gillett, 1975), no other region of the world contains as much diversity. The species differ in leaf shape (from simple to compound to highly dissected), flower head size and number, achene size and shape (from flat and straight to highly coiled) and presence and type of dispersal mechanism (awns of various lengths and shapes, pubescence, and presence or absence of wings). They vary in growth form from small trees over 2 m tall with a woody trunk to tall shrubs to erect and prostrate herbaceous forms. Differences between species in all these characters are maintained under standard growing conditions, which demonstrates that they are under strong genetic control. Ecological differentiation has occurred as well, since different species are found in environments as disparate as coastal sand dunes, montane bogs, arid cinder cones, open grassy areas and rain forests.

On the other hand, all species examined are chromosomally similar, with a diploid number of 72 (Skottsberg, 1953; Gillett and Lim, 1970). Since the base number for Bidens is 12 , all Hawaiian species are hexaploid. Moreover, all interspecific crosses attempted between Hawaiian species result in fertile hybrids (Gillett, 1972a, 1972b, 1973; Gillett and Lim, 1970). Attempted crosses involving B. cosmoides were reported to be unsuccessful by Gillett (1975) but B. cosmoides has been intercrossed successfully with other species by Ganders (pers. comm.). Crosses between Hawaiian and Marquesan species also produce viable although sterile progeny, but the Polynesian taxa will not hybridize with B. pilosa, an American species often compared with them (Gillett, 1972b, 1973). Natural hybridization has been documented in nature between at least three pairs of species that are parapatric in distribution (Degener, 1946; Mensch and Gillett, 1972; Sherff, 1937; Ganders, pers. comm.), but in general taxa do not interbreed because they occur in different habitats.

Experimental crosses between taxa and subsequent selffertilization of progeny have revealed that many of the morphological differences between species may be controlled by a relatively small number of genes. Leaf shape, awn length and stem pigmentation all seem to be under polygenic control because of the continuum of variation observed in the $F_{z}$ generation, but parental forms appear in the $F_{2}$ generation and minimum estimates of the number of genes involved using the Sinnott et al. (1950) formula gave a value of one for the latter two characters.

Intraplant variation prevented quantitative analysis of the genetic basis of leaf form (Gillett and Lim, 1970; Mensch and Gillett, 1972).

The information presented above leads to opposing predictions about the genetic differentiation of Bidens on the Hawaiian Islands. The extent of morphological and ecological differentiation suggests that the taxa are highly differentiated genetically. However, the chromosomal similarity of species, absence of genetic isolating mechanisms, and the possibility that many morphological characters may be controlled by very few genes suggests that genetic differences among the taxa may be limited to a small portion of the genome and may not reflect overall genetic differentiation. The taxa may be distinct only in certain morphological and ecological characters of adaptive significance commonly used by taxonomists.

### 1.3 Genetic Differentiation Of Plant And Animal Populations.

Research during the first years of the analysis of isozyme variation in natural populations suggested two generalizations regarding the genetic relationships of populations. First, there was a correlation between genetic similarity of populations and taxonomic rank: closely related taxa were more similar genetically than more distantly related ones. This was evident at the subspecific level in the Drosophila willistoni group (Ayala et al., 1974) and in various vertebrate species as
well as at the level of species and genera in various animal groups (Avise, 1976). Gottlieb (1977) found the same correlation in a survey of studies involving higher plants, with different species being on average only about two-thirds as similar genetically as conspecific populations.

Similarity values much higher than the average were obtained in comparisons of species pairs that differed in very few morphological characters. Although taxonomic relationship of organisms usually reflects the degree of morphological similarity between them, species may be named on the basis of very few morphological differences if a reliable discontinuity in variation exists and the species are genetically isolated. In the genera Clarkia, Gaura, and Stephanomeria, the species pairs examined electrophoretically are reproductively isolated and the morphological differences between them are very small, consisting of only one or two characters (Gottlieb, 1977). Thus, a second generalization was that there seemed to be a correlation between the degree of morphological and genetic similarity. This correlation was also apparent in animals. When species within the genera Drosophila, Lepomis, and Peromyscus were arranged according to genetic similarity, the resulting classification was very similar to that produced on the basis of morphological criteria alone (Avise, 1974).

Subsequent studies of animals revealed numerous exceptions to both correlations (Avise et. al., 1975; Kornfield and Koehn, 1975; Carson et. al., 1975; Nixon and Taylor, 1977; Mitton and Koehn, 1975) so that neither now seems tenable. In plants,
however, they have generally been upheld, although there is no apparent correlation between morphological and genetic differences among species of Capsicum (Jensen et al., 1979), morphologically distinct species of Sullivantia cannot be distinguished by their isozymes (Soltis, 1982), and morphological differentiation is exceeded by genetic differentiation in subspecies of Coreopsis cyclocarpa (Crawford and Bayer, 1981). The plant groups in which genetic and morphological differences are generally well-correlated or in which genetic similarity is related to taxonomic rank include Coreopsis (Crawford and Bayer, 1981; Crawford and Smith, 1982), Sabatia (Bell and Lester, 1978), Solanum (Whalen, 1979), Capsicum (McLeod et al., 1979), Machaeranthera (Arnold and Jackson, 1978, 1979), Typha (Mashburn et al., 1978; Sharitz et al., 1980), Brassica (Yodava et al., 1979), Chenopodium (Crawford, 1979; Crawford and Wilson, 1979), Phlox (Levin, 1978), Plantago (van Dijk and van Delden, 1981), Hordeum jubatum (Shumaker and Babbel, 1980), Desmodium nudiflorum (Schaal and Smith, 1980), and Chondrilla juncea (Burdon et al., 1980). The correlation is also present at the subpopulation level in populations of Veronica peregrina (Keeler, 1977). It is not known, however, whether the correlation of morphological and genetic differentiation in plants holds on the scale of a recent and extensive adaptive radiation. Gottlieb (1976) concluded that the speciation process in many annual diploid plants occurs prior to the acquisition of distinct adaptations and is largely fortuitous, based on studies of Clarkia, Gaura, and

Stephanomeria. Species in these genera are reproductively isolated because of chromosomal differences, but divergence at isozyme loci has not yet occurred. However, Hawaiian Bidens are perennial and differ from the annuals because they exhibit extensive morphological and ecological differences among taxa and lack reproductive isolating mechanisms. It is therefore of considerable interest to see whether electrophoretically detectable genetic differentiation has occurred among the Hawaiian taxa of Bidens.

### 1.4 Electrophoresis And Isozymes

Electrophoresis essentially consists of subjecting plant or animal extracts to an electric current and separating molecules as they migrate through a gel or other medium on the basis of electrostatic charge, size and shape. In starch gel electrophoresis of enzymes, the molecules are separated mainly on the basis of differences in net charge determined by the number of charged amino acids they contain. The positions of the enzymes on the gel are identified using histochemical staining techniques (Hunter and Markert, 1957). The staining methods couple the enzyme-substrate reaction with a reaction producing insoluble pigment visible in the gel, thus marking the positions to which the enzymes migrated. Mobility differences of enzymes indicate differences in the alleles producing them, but identical migration rates are not necessarily evidence of identical alleles. Approximately $30 \%$ of nucleotide base
substitutions do not affect amino acid sequences of proteins (Selander, 1976), and many amino acid substitutions do not affect the net electrostatic charge of enzymes because only 4 of the 20 common amino acids are charged in the pH ranges usually employed in electrophoresis. Other differences can be demonstrated in a group of enzymes migrating to the same position on a gel, such as differences in heat stability (Bernstein et al., 1973). King and Wilson (1975) estimated that only $27 \%$ of point mutations are electrophoretically detectable. A further limitation of electrophoresis is that only structural genes, which make up less than $10 \%$ of the eukaryotic genome, can be surveyed (Selander, 1976).

In spite of these limitations, gel electrophoresis represents a major advance in surveying populations genetically. Genetic analysis of populations using only those genes inferred from morphological data is inherently biased because only polymorphic genes producing variant phenotypes can be identified. Examination of gene products permits consideration of monomorphic loci as well, since the technique depends simply on production of functional enzymes rather than upon the production of different ones. Polymorphism at a locus is inferred from the number, position and pattern of bands instead of being a prerequisite for identification of a locus. Morphological characters may be determined not only by a large number of genes but by the environment as well, which makes genetic analysis difficult. Furthermore, simultaneous examination of many morphological characters is complicated by
the pleiotropic effects of some genes. A direct correspondence exists between enzyme loci and the polypeptides they produce, allowing unambiguous interpretation of phenotypes. Their genetic control is generally well-documented, moreover, in contrast to the scarcity of genetic information regarding morphological characters.

It is not known whether isozyme loci are the unbiased sample of the genome that they were originally hoped to be. Even if extrapolation to the entire genome is not possible, these genes are a part of the genome not previously accessible to population biologists and conclusions derived from their study are of as much value as those based on any other subset of loci. The large number of genes made available for investigation by electrophoresis and the quantitative estimates of allele frequencies derived for each locus allow precise description and comparison of populations, advantages not shared by other techniques.
II. MATERIALS AND METHODS

### 2.1 Population Samples

Plants of the endemic Hawaiian taxa were collected either as seeds or cuttings from natural populations. Table 1 lists population localities, sample sizes and the type of material collected. Figures 2-4 show the locations of the populations in the Hawaiian Islands. Table 2 gives similar information for the American species, and also indicates their geographical distribution.

Seed samples of populations comprise the majority of material examined in this study, but, unfortunately, detailed information about many of the seed collections is lacking. Several collectors provided seed samples, and often the seeds representing $a$ taxon in a certain locality were a bulk collection without indication of the number of plants from which they were gathered. It is conceivable that a large seed sample could be the progeny of a single self-fertilized individual, and therefore perhaps not representative of the population. It is impossible to know the extent of sampling bias in these cases.

The larger collections of cuttings or seed families were collected either from all individuals in a population or from a random sample, although sampling bias may be involved in localities at higher elevations where the steep terrain can make plants difficult to find or almost impossible to reach.
table 1. collections of hawaitan taxa studied.

| TAXON | ACRONYM | POPULATION <br> NUMBER | $\begin{aligned} & \text { SAMPLE } \\ & \text { SIZE } \end{aligned}$ | TYPE OF MATERIAL ${ }^{1}$ | LOCALITY |
| :---: | :---: | :---: | :---: | :---: | :---: |
| B. asymmetrica | ASYM | $\begin{aligned} & \text { B4 } \\ & 890 \end{aligned}$ | $\begin{aligned} & 27 \\ & 11 \end{aligned}$ | $\begin{gathered} S(5), P(3) \\ S, P(9) \end{gathered}$ | Manoa Cliffs Trail, Oahu Aiea Ridge, Oahu |
| B. cervicata | CERV | $\begin{aligned} & 88 \\ & 883 \\ & 887 \\ & 888 \end{aligned}$ | $\begin{array}{r} 13 \\ 1 \\ 38 \\ 13 \end{array}$ | $\begin{aligned} & S(\geq 2) \\ & P \\ & S(\geq 4) \\ & S(1) \end{aligned}$ | Nualolo Valley, Kauai Makaha Ridge, Kauai Ohikilolo Ridge, Oahu Ohikilolo Ridge, Oahu |
| B. forbesif ssp. forbesii | FORE F | $\begin{aligned} & \text { B } 12 \\ & \text { B } 13 \\ & \text { B14 } \\ & \text { B74 } \end{aligned}$ | $\begin{array}{r} 9 \\ 2 \\ 25 \\ 2 \end{array}$ | $\begin{aligned} & S(\geq 7), P(1) \\ & S(7), P(2) \\ & P \end{aligned}$ | Haena Dry Cave, Kaua Hanalei Bay, Kauai Lumahai Beach, Kaua <br> Na Pali Coast, Kaua |
| B. hawaiensis | HAWA | $\begin{aligned} & \text { B48 } \\ & \text { B50 } \\ & \text { B231 } \end{aligned}$ | $\begin{array}{r} 10 \\ 52 \\ 137 \end{array}$ | $\begin{gathered} S(1), P(3) \\ S(7), P(6) \\ S(\geq 8) \end{gathered}$ | Kehena, Hawai <br> Kaimu, Hawaii <br> Kalapana, Hawaii |
| B. mauiensis | maui | $\begin{aligned} & \text { B } 10 \\ & \text { B27 } \\ & \text { B126 } \\ & \text { B129 } \end{aligned}$ | $\begin{array}{r} 16 \\ 3 \\ 4 \\ 3 \end{array}$ | $\begin{gathered} S(\geq 6) \\ S \\ S \\ P \end{gathered}$ | Waiehu, Maui <br> Z.OO. Maui <br> Ukumehame, Maui <br> Awalua Gulch, Lanai |
| B. meriziesii ssp. filiformis | MENZ F | $\begin{aligned} & \text { B } 109 \\ & \text { B130 } \\ & \text { B218 } \\ & \text { B219 } \\ & \text { B224 } \\ & \text { B238 } \end{aligned}$ | $\begin{array}{r} 48 \\ 57 \\ 100 \\ 61 \\ 7 \\ 14 \end{array}$ | $\begin{gathered} S(\geq 7) \\ S \\ S(36) \\ s(30) \\ s \\ s \end{gathered}$ | Nohonaohae. Hawai <br> Kipuka Kalawamauna, Hawai; <br> Puu Ahumoa. Hawai <br> Puu Koko, Hawaii <br> Puu Kanalopakanui, Hawaii <br> Puu Waawaa, Hawaii |
| B. micrantha ssp. micrantha | MICR M | $\begin{aligned} & 824 \\ & \text { B25 } \\ & \text { B78 } \\ & \text { B80 } \\ & \text { B133 } \end{aligned}$ | $\begin{array}{r} 3 \\ 2 \\ 21 \\ 2 \\ 12 \end{array}$ | $\begin{gathered} P \\ P \\ S(4) \\ S \\ S \end{gathered}$ | Iao Valley, Maui <br> Kahoma Ditch Trail, Maui <br> Wailuku, Maui <br> Zoo, Maui <br> Honokowai Ditch Trail, Maúi |


| TAXON | ACRONYM | POPULATION NUMBER | $\begin{aligned} & \text { SAMPLE } \\ & \text { SIZE } \end{aligned}$ | TYPE OF MATERIAL' | locality |
| :---: | :---: | :---: | :---: | :---: | :---: |
| B. micrantha ssp. ctenophylla | MICR C | B149 | 80 | $S(\geq 10)$ | Kona, Hawaii |
| B. Micrantha ssp. kalealaha | MICR K | $\begin{aligned} & 891 \\ & 8197 \end{aligned}$ | $2{ }^{1}$ | $\stackrel{S}{s(\geq 2)}$ | Kahikinui, Maui Kapalaoa Cabin. Maui |
| B. molokaiensis | MOLO | $\begin{aligned} & 811 \\ & \text { B72 } \end{aligned}$ | $\begin{array}{r} 2 \\ 62 \end{array}$ | $S^{P}(5)$ | Diamond Head, Oahu Hoolehua, Molokai |
| B. populifolia | POPU | $\begin{aligned} & \text { B42 } \\ & \text { B11 } \end{aligned}$ | 18 1 | P | Kahana Valley, Oahu Kaawa, Oahu |
| B. sandvicensis ssp sandvicensis | SAND S | $\begin{aligned} & \text { B35 } \\ & \text { B44 } \\ & \text { B111 } \\ & \text { B112 } \\ & \text { B116 } \\ & \text { B200 } \end{aligned}$ | $\begin{array}{r} 9 \\ 21 \\ 2 \\ 8 \\ 5 \\ 79 \end{array}$ | $\begin{gathered} P \\ S(5) \\ S(2) \\ S(12) \\ S(2) \\ S(\geq 9), P(33) \end{gathered}$ | Nuuanu Pali, Oanu Lanipo Trail, Oahu Kalepa Summit, Kauai Wailua, Kauai Haiku Valley, Oanu Wahila Ridge, Oahu |
| B. sandvicensis ssp. confusa | SAND C | $\begin{aligned} & 833 \\ & 834 \end{aligned}$ | $\begin{array}{r} 2 \\ 21 \end{array}$ | $S_{S}^{P}(3)$ | Waimea Canyon, Kauai Puu Ka Pele, Kauai |
| B. torta | TORT | $\begin{aligned} & \text { B15 } \\ & \text { B37 } \\ & \text { B89 } \\ & \text { B110 } \\ & \text { B213 } \\ & \text { B215 } \\ & \text { B257 } \end{aligned}$ | $\begin{array}{r} 79 \\ 64 \\ 19 \\ 3 \\ 110 \\ 5 \\ 49 \end{array}$ | $\begin{gathered} S(2) \\ S(1), P(60) \\ S(4) \\ S \\ S(\geq 8), P(8) \\ S \\ S(6) \end{gathered}$ | Pahole Gulch, Oahu Palikea Trail, Oahu Ohikilolo Ridge, Oahu Kawai-Iki Diteh Trail, Oahu Mount Kaala, Oanu Kolekole Pass, Oahu Waianae Kai, Oahu |
| B. wiebkei | WIEB | B259 | 35 | $s(\geq 5)$ | Halawaiki Gulch, Molokai |

[^0]KAUAI


Figure 2. Locations of populations surveyed on Kauai and Oahu.

MOLOKAI


Figure 3. Locations of populations surveyed on Maui and Molokai.

LANAI

HAWAll


Figure 4. Locations of populations surveyed on Hawaii and Lanai.

TABLE 2. COLLECTIONS OF AMERICAN SPECIES.

| TAXON | $\begin{aligned} & \text { SAMPLE } \\ & \text { SIZE } \end{aligned}$ | MATERIAL COLLECTED ${ }^{1}$ | LOCALITY | DISTRIBUTION ${ }^{2}$ |
| :---: | :---: | :---: | :---: | :---: |
| B. amplissima | 4 C | P | Jericho Lake. Vancouver, British Columbia | Reputedly endemic to Vancouver Island but also found in the lower mainland of B.C. |
| B. cynapifolia | 10 | S | Hanauma Bay, Oahu | Native to the West Indes and continental tropical America An introduced weed in Hawai $i$ first collected in 1929 on the island of Hawaii. ${ }^{3}$ |
| B. frondosa | 17 | $p$ | Spanaway Lake. Pierce County, Washington | Newfoundland and Nova Scotia to Washington, and south to Louisiana, Virginia, and California. |
| B. tripartita | 10 | $p$ | Jericho Lake. Vancouver. <br> British Columbia | A nearly cosmopolitan north temperate weed native to eastern North America and Europe. |

${ }^{1} \mathrm{P}$ refers to wild collected plants and $S$ to seeds.
${ }^{2} \mathrm{Hitchcock}$ et al. (1955)
${ }^{3}$ Degener (1946).

On the other hand, some of the smaller collections of cuttings or seed families may in fact be representative because the populations were so small. As an extreme example, the only population of Bidens molokaiensis on Oahu consisted of seven mature plants and seven seedlings in 1979. A sample of two cuttings, which were homozygous and identical at all isozyme loci studied, probably provides a completely representative sample of the taxon on this island.

### 2.2 Growth Of Plant Material

Seeds were planted in vermiculite and grown in growth chambers with 14 hours light at $25^{\circ} \mathrm{C}$ and 10 hours dark at $15^{\circ} \mathrm{C}$, and transplanted into soil about three weeks after emergence. Cuttings were dipped in rooting hormone and maintained under mist until well rooted. They were then planted in soil and grown in greenhouses at the University of British Columbia.

### 2.3 Electrophoresis

Horizontal starch gel electrophoresis was used to assay isoenzymes. The methods used generally follow Layton (1980), with some modifications of the gel recipes, composition of buffer solutions and running conditions (Tables 3 and 4). Most of the study was done using Electrostarch Lot \#307 but Lot \#392
table 3. RUNNing CONDitions used for enzyme systems.

| ENZYME | EXTRACTION BUFFER | GEL COMPOSITION ${ }^{1}$ (\%w/v) | ELECTRODE BUFFER | CURRENT <br> or voltage |
| :---: | :---: | :---: | :---: | :---: |
| Malate dehydrogenase MDH E.C.1.1.1.37 | A | gel buffer A $12.5 \%$ starch $20 \%$ sucrose | A | 350 V |
| ```Phosphoglucose isomerase PGI E.C.5.3.1.9 Phosphoglucomutase PGM E.C.2.7.5.1 Malic enzyme ME E.C.1.1.1.40``` | A | ge) buffer B 12.5\% starch 10\% sucrose | B | 350 V |
| ```\beta-Glucosidase GLU E.C.3.2.1.21 Hexose aminidase HA E.C.3.2.1.30 x dehydrogenase xDH``` | A | gel buffer $C$ 12.5\% starch 10\% sucrose | C | 350 V |
| ```Diaphorase DIA E.C.1.6.4.3 Leucine aminopeptidase LAP E.C.3.4.1.1``` | B | gel buffer D 12.5\% starch ${ }^{2}$ $10 \%$ sucrose | D | 75 mA |

${ }^{1}$ Gels were made the morning of a run with Lot $\# 307$, but the evening before with lot $\# 392$. ${ }^{2} 14.3 \%$ starch used with Lot $\# 392$.

TABLE 4. COMPOSITION OF BUFFER SOLUTIONS.

${ }^{1} \mathrm{pH}$ of solutions adjusted with NaOH or HCl unless otherwise noted.
${ }^{2}$ Some buffer solutions are slightly modified from these references.
was also used, which required modified methods of preparation to achieve similar results (Table 3 ). The addition of sucrose to the gels improved resolution of the bands. With Lot \#307 starch was cooked, degassed, poured into $150 \times 200 \times 10 \mathrm{~mm}$ plexiglass gel molds, wrapped in plastic and stored in the refrigerator the morning of a run.

About 8 mg of young leaf tissue was ground on ice (to inhibit enzyme activity) with one drop of extraction buffer and 4mg polyvinylpolypyrrolidone in spot plates. The homogenate was absorbed onto $9 \times 5 \mathrm{~mm}$ wicks cut from Whatman 3 MM chromatographic paper, and the wicks inserted into slots cut 30 mm from the end of the gel. Migration of enzymes was monitored with dilute red food colouring absorbed onto wicks at either end of the group of samples. Results were standardized by alwaỳs including one of three plants of known genotype in the run.

Electrophoresis was performed in a refrigerator maintained at $0-4^{\circ} \mathrm{C}$ to avoid overheating which distorts migration and denatures enzymes. Gels were subjected to electric current for about four hours, using J-Cloths to complete the circuit between the ends of the gel and the electrode trays containing an electrode and buffer solution. All but one of the isozymes (a PGI-5 variant) migrated anodally in the conditions employed (i.e., toward the positive electrode).

Gels were sliced with Gibson .008 plain steel ball end guitar string guided by 1.5 mm thick plexiglass strips placed on either side of the gel. Top and bottom slices were discarded and the rest stained for appropriate enzymes using histochemical
staining methods listed in Table 5. (Enzyme abbreviations are listed in Table 3.) In most cases, gels were bathed in 60 mls of stain solution, but with GLU and HA 5 mls of stain solution were poured on top of the gel. Most of the stains depended on reactions producing insoluble products visible in natural light, but GLU and HA bands were only visible under long-wave UV light and had to be scored before the bands diffused over the surface of the gel. In attempting to stain for ADH (alcohol dehydrogenase) the appropriate bands rarely appeared. Instead, a much more slowly developing band appeared overnight for each individual, sometimes even in the absence of ethanol (but not in the absence of a sample). This mystery enzyme was accordingly termed xDH.

After the staining reaction was complete, PGI, PGM and MDH gels were fixed in a 1:1 glycerine-water solution (Siciliano and Shaw, 1976) and the rest in a 1:4:5 acetic acid-methanol-water solution (Allendorf et al., 1977) to preserve resolution.

### 2.4 Enzymes Studied

The choice of enzyme systems used to estimate genetic variability affects the results obtained. Enzyme polymorphism has been shown to be correlated among enzymes of the glycolyticKrebs cycle (Sing and Brewer, 1971). Johnson (1974) has suggested positive relationships between enzyme polymorphism and regulatory character, and between the variability of enzymes and the variability of their substrates. Harris et al. (1977)

TABLE 5. STAINING SOLUTIONS EMPLOYED FOR ENZYME SYSTEMS.

| ENZYME | STAIN RECIPE | REFERENCE ${ }^{2}$ |
| :---: | :---: | :---: |
| MDH | 200mM Tris-HCl, pH 8.0 80 mM DL-malic acid <br> 0.6 mM NAD <br> 0.2 mM MTT <br> 0.2 mM NBT <br> 0.25 mM PMS | ```Siciliano & Shaw (1976)``` |
| PGI | 130 mM Tris-HC1, pH 8.0 <br> 5 mM MgCl <br> $0.4 \mathrm{U} / \mathrm{m} 1$ Glucose-6-phosphate dehyarogenase <br> 0.75 mM D-fructose-6-phosphate <br> 0.2 mM NADP <br> 0.2 mM MTT <br> 0.25 mM PMS | $\begin{gathered} \text { Roose \& Gottlieb } \\ (1976) \end{gathered}$ |
| PGM | ```130mM Tris-HCl, pH 8.0 5mM MgCl 5.5mM -D-glucose-1-phosphate (Na salt) 0.004mM -D-glucose-1-phosphate (K salt) 0.4U/ml Glucose-6-phosphate dehydrogenase 0.2mM NADP O.3mM MTT 0.25mM PMS``` | $\begin{gathered} \text { Roose \& Gottlieb } \\ (1976) \end{gathered}$ |
| ME | ```200mM Tris-HCl, pH 8.0 5mM MgCl 25mM L-malic acid 0.2mM NADP 0.3mM MTT 0.25mM PMS``` | Siciliano \& Shaw (1976) |
| GLU | 50 mM Citrate-phosphate, pH 4.0 6 mM 4 -methylumbelliferyl- $\boldsymbol{\beta}$ -D-glucoside | $\begin{gathered} \text { Yeh \& Layton } \\ (1979) \end{gathered}$ |
| HA | 25mM Citrate-phosphate, pH 4.0 5 mM 4 -methylumbelliferyl-N-acetyl-$\beta-D-g l u c o s a m i n i d e$ | $\begin{gathered} \text { Siciliano \& Shaw } \\ (1976) \end{gathered}$ |
| $\times \mathrm{DH}$ | 200mM Tris-HC1, pH 8.5 $4 \%$ 95\% ethanol <br> 0.6 mM NAD <br> 0.2 mM MTT <br> 0.2 mM NBT <br> 0.25 mM PMS | $\begin{gathered} \text { Siciliano \& Shaw } \\ (1976) \end{gathered}$ |
| DIA | ```200mM Tris-HCl, pH 8.5 0.06mM 2,6-dichlorophenol-indophenol 0.06mM NADH O.2mM MTT``` | $\begin{gathered} \text { Yeh \& o'malley } \\ (1980) \end{gathered}$ |
| LAP | presoak solution 500 mM Boric acid 5 mM MgCl | $\begin{gathered} \text { Brewer \& Sing } \\ (1970) \end{gathered}$ |
|  | ```stain solution, pH 5.2 2OmM Tris-HCl 20mM Malic acid 0.65mM L-leucyl- }\boldsymbol{\beta}\mathrm{ -naphthylamide HCl 0.0006% Fast black K salt (w/v)``` |  |

[^1]found that enzymes composed of more subunits have fewer variants in humans, and ward (1977) found a similar correlation between quaternary structure and polymorphism of enzymes in many vertebrate and invertebrate species. There may also be a positive correlation between variability and subunit molecular weight (Koehn and Eanes, 1977; but see Johnson, 1977, for an opposite view).

Siciliano and Shaw (1976) pointed out that out of the approximately 1000 enzymes which have been identified, histochemical staining techniques are available for fewer than 50. Twenty-seven of these were attempted for Bidens but enzyme activity was not recovered for all of them. Furthermore, adequate resolution of bands was not achieved for many enzyme systems despite the various types and combinations of extraction, gel and electrode buffers and gel composition tested. Finally, chemical differences among the Hawaian taxa resulted in a further reduction of usable systems because of the inability of any one method to achieve good resolution in all populations. Using different techniques for different taxa would have made it difficult to establish homology of bands. This problem eliminated the use of acid phosphatase, glutamate oxaloacetate transaminase, and certain regions of MDH and DIA. The nine enzyme systems finally used to compare the Hawaian populations of Bidens were thus chosen not with due consideration of structural and functional constraints, but purely on the basis of what systems worked.

As with all electrophoretic studies, it is hard to know how
representative this subset of enzymes is of structural genes or of the genome in general. The number of enzymes studied is about average for plant population studies but the number of loci is well above average. Fortunately, most of these loci (17 of 24) code for enzymes examined in over $65 \%$ of previous studies of plant populations (Gottlieb, 1981), so that comparisons with other plants are possible.
III. INHERITANCE OF ISOZYMES

### 3.1 Definitions And Nomenclature

The terms allozyme and isozyme were presumably conceived to distinguish between enzymes that are allelic and those that are not. Thus, for enzymes consisting of one polypeptide (monomeric enzymes) the products of a heterozygous locus would be allozymes while products of different loci would be called isozymes. Multimeric enzymes pose a problem, however, because it is associations of gene products rather than the gene products themselves that are being compared. An enzyme composed of two unlike subunits coded by different loci (an interlocus heterodimer) is both allelic and non-allelic to an enzyme composed of identical subunits coded by an allele at one of the loci. The convention is to call these isozymes (Gottlieb, 1981) but this makes allozymes very rare indeed when several genes are involved. Because even the monomeric enzymes that show polymorphism in Bidens are controlled by more than one locus, I will collectively call the multiple forms of an enzyme system isozymes, ignoring the small subsets of molecules which could be distinguished as allozymes.

Table 6 lists for each enzyme the substructure, number of loci apparently controlling it and the number of loci scored for this study. The nomenclatural convention used in the following sections is to label loci numerically with the most anodally
table 6. enzyme suestructure, genetic control and number of LOCI SCORED.

| ENZYME | - substructure. | Number of LOCI EXPRESSED | NUMBER OF LOCI SCORED |
| :---: | :---: | :---: | :---: |
| MDH | dimeric | $\geq 8$ |  |
| PGI | dimeric | - 5 | 6 |
| PGM | monomeric | 4 | 4 |
| ME | tetrameric | $\geq 2$ | 1 |
| GLU |  | $\geq 2$ | , |
| HA $\times \mathrm{DH}$ |  | $\geq 2$ | 1 |
| XDIA |  | 1 | 1 |
| LAP | monomeric monomeric | $\geq 4$ | 2 |
|  |  | 2 | 2 |

migrating locus for each enzyme system as "1" and the alleles alphabetically with the most anodally migrating allele at each locus as "a", with the rest proceeding in sequence according to position on the gel.

### 3.2 Types Of Evidence

Many lines of evidence can be used to infer the genetic control of enzyme systems. The most fundamental of these is genetic analysis, the crossing of individuals with different band patterns and examining segregation in the progeny. The number of loci involved and the number of alleles at each can be deduced from a large number of such crosses. This is a timeconsuming procedure in Bidens, in which florets are small, difficult to emasculate, and only produce one achene each. Although the ultimate test of the genetic hypothesis can only be of this nature, there are fortunately easier ways to arrive at the actual inferences.

The most useful of these is knowing the substructure of the enzyme. Alleles code for polypeptides which may be functional as monomeric molecules (e.g., LAP and PGM) or may need to bind to other polypeptides to function. Such multimeric enzymes are often dimeric (e.g., MDH and PGI), but tetramers such as catalase (Scandalios, 1965), hexamers such as glutamate dehydrogenase (Goldin and Frieden, 1971), and others also exist. While it is the composition of the allele product that affects
electrophoretic mobility and thus the position of bands on the gel, it is the substructure of the enzyme which determines the number of bands and their relative intensity. A heterozygote at one locus will produce two bands for a monomeric enzyme but three for a dimeric one: the additional band represents the association of unlike subunits (produced by the different alleles) and has a mobility intermediate to that of the molecules formed by the association of like subunits (coded by the same allele). Because of the presumably random association of subunits, molecules consisting of unlike subunits will be twice as common as either of the homodimers, resulting in a 1:2:1 ratio of band intensities. When more than one locus is involved patterns are more complex, but a brief examination of band patterns produced by individuals in a population still affords a clue to the genetic control of the enzyme system. The literature concerning enzymes and their use in population studies is also helpful in elucidating the inheritance of isozymes. In addition to general agreement in substructure for a given enzyme in different higher plant species, there are also similarities in the number of loci coding for each enzyme at a given ploidy level (Gottlieb, 1982). Because Hawaiian Bidens are hexaploid (Gillett and Lim, 1970), one would expect more loci than usual to be controlling each enzyme system.

Analysis of haploid tissue can simplify band patterns because of the presence of only one allele per locus. This is especially valuable in conifers where megagametophytes are large
enough to be studied individually. In angiosperms an extract of pollen produced by a plant can be run instead. This is less useful because of the lumping of many haploid genotypes, but heteromeric enzymes will not be formed through the association of subunits produced by different alleles of the same locus. A comparison of pollen and leaf tissue patterns can reveal which bands are composed of such heteromers, and can enable assignment of alleles to certain loci. This technique did not prove very useful with Bidens because of the small number of plants in flower, faint staining of enzymes from pollen samples, and differences in the loci expressed in pollen and leaf tissue. Only PGI, PGM and MDH showed any activity at all in pollen samples, and in $M D H$ an additional locus not active in leaves complicated the analysis. Because many genes code for each of these enzymes, only the pollen and leaf comparisons of double heterozygotes would have yielded information, and these were not encountered in the plants sampled.

PGI, PGM and MDH isozymes are compartmentalized within the cell (Gottlieb, 1982). Although all are produced by nuclear genes, the products of some loci are found in plastids and others in the cytoplasm. Furthermore, there is no interaction between the polypeptides of plastid and cytoplasmic enzymes so that heterodimers are not formed between the subunits produced by the different loci. Because the plastid forms of PGI and PGM migrate to a different region of the gel, genetic analysis simply treats each region separately. The compartmentalization of MDH in Bidens may not be reflected in separation of regions,
so that comparison of patterns produced by chloroplast fractions and unfractionated leaf samples might help elucidate the genetic control. The results were ambiguous, however, so the attempt to look at chloroplast isozymes was dropped.

### 3.3 Monomorphic Enzymes

Staining for $\mathrm{ME}, \mathrm{xDH}, \mathrm{GLU}$ and HA resulted in an identical, single band for all individuals sampled from seedlings and cuttings. The simplest interpretation of this is that each enzyme is controlled by one homozygous locus, with all plants sharing the same allele. Adult plants occasionally had other bands for ME, GLU and HA, suggesting that more than one locus codes for these systems although only one is expressed in younger plants.

### 3.4 Polymorphic Enzyme Systems

Several of the polymorphic enzymes had variants which migrated to two distinct regions of the gel. The zones of activity did not show any correlated variation and were therefore treated independently with respect to genetic hypotheses, each zone being considered to be controlled by different loci.

Because of small variation among runs in the mobility of
bands, alleles were accepted as being different only if there was a large and consistent mobility difference between bands or if heterozygotes were found for alleles coding for polypeptides with small mobility differences. Null alleles were invoked in preference to inferring several identical uncommon alleles for different loci. The assumption that a given allele would be found at one locus only without proof to the contrary seems reasonable because of the small probability of independent mutations at two loci having exactly the same phenotypic effect. The most common allele at two PGI loci was in fact shared, but there is strong evidence for this in the band intensity patterns. This is not too surprising for a common allele given the polyploid status of Hawaiian Bidens in which loci are presumably duplicated.

When more than one locus controlled a region of activity, alleles were assigned to them by finding a genotype homozygous for the common allele at one locus and heterozygous at the other. Finding such heterozygotes for each allele permitted assignment of all alleles to loci.

Evidence for the genetic hypotheses for all polymorphic loci is given in Table 7. Although sample sizes are small, the data generally conform to Mendelian expectations. Crosses involving null alleles show a pronounced lack of homozygous null progeny, however. This is not unexpected because genotypes unable to produce functional enzymes would not be likely to survive long enough to be studied electrophoretically.

TABLE 7. GENETIC ANALYSIS OF ELECTROPHORETIC VARIANTS.

| LOCUS | PARENTAL PHENOTYPES |  | bc | cc | bd | OFFSPRING PHENOTYPES |  |  |  |  | 99 | an | bn | $n \mathrm{n}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | bb |  |  |  | cd | dd | de | ee | $d g$ |  |  |  |  |
| PGI-4 | dd $x$ dd |  |  |  |  |  | 10 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 4 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 7 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 10 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 9 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 10 |  |  |  |  |  |  |  |
|  |  |  |  | - |  |  | 9 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 10 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 16 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 2 |  |  |  |  |  |  |  |
|  | . |  |  |  |  |  | 26 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | $1!$ |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 12 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 10 | : |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 24 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | 4 |  |  |  |  |  |  |  |
|  | $\cdots \mathrm{cd} \times \mathrm{cd}$ |  |  | 2 |  | 3 | 1 |  |  |  |  |  |  |  |
|  |  |  |  | 4 |  | 6 | 11 |  |  |  |  |  |  | - |
|  |  |  |  | 5 |  | 10 | 1 |  |  |  |  |  |  |  |
|  | $\operatorname{dg} x \mathrm{dg}$ |  |  |  |  |  |  | - |  | 3 | 3 |  |  |  |
|  | dg $x$ dg |  |  |  |  |  |  |  |  | 1 | 5 |  |  | . |
| PGI-5 | $\operatorname{cc} \times \mathrm{cc}$ |  |  | 24 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 10 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 4 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 7 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 9 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 8 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 10 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 10 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 24 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 26 |  |  |  |  |  |  |  |  | . |  |
|  |  |  |  | 2 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 16 |  |  |  |  |  |  |  |  |  |  |
|  |  |  | - | 4 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 16 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 10 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 12 |  |  |  |  |  |  |  |  |  |  |
|  | $\mathrm{cd} \times \mathrm{cd}$ |  |  | 2 |  | 3 | 1 |  |  |  |  |  |  |  |
|  |  |  |  | 1 |  | 6 | 2 |  |  |  |  |  |  |  |
|  |  |  |  | 3 |  | 5 | 3 |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| PGM-1 | $b b \times b b$ | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  | . |  |  |  |  |
|  |  | 20 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 16 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 24 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 12 |  |  |  |  |  |  |  |  |  |  |  |  |
|  | $\operatorname{cc} \times \mathrm{cc}$ |  |  | 9 |  |  |  |  |  |  |  |  |  |  |
|  | bod $x$ bd | 2 | - |  | 1 |  | 5 |  |  |  |  |  |  |  |
|  | bn $X$ bn | 2 |  |  |  |  |  |  |  |  |  |  | 5 |  |
|  |  | 1 |  |  |  | . |  |  |  |  |  |  | 2 | 1 |
|  |  | 3 |  |  |  |  |  |  |  |  |  |  | 8 |  |

TABLE 7 cont.

|  | PARENTAL |  |  |  |  | OFF | RI | PHE | TYP |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LOCUS | PHENOTYPES | aa | $a b$ | bo | ac | bc | cc | ad | bd | cd | dd | an | bn | nn |


41
$3 \quad 3$
bn $x$ bn
$\begin{array}{ll}4 & 5 \\ 1 & 2\end{array}$

$b b \times b b$

TABLE 7 cont.

| LOCUS | PARENTAL PHENOTYPES | aa | $a b$ | bb | ac | OFFSPRING PHENOTYPES |  |  |  |  | dd | an | bn | $n \mathrm{n}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  | bc | cc | ad | bd | cd |  |  |  |  |
| MDH-2 | ad $X$ ad dd $X$ dd | $\begin{aligned} & 4 \\ & 1 \end{aligned}$ |  | . |  |  |  | $\begin{aligned} & 8 \\ & 5 \end{aligned}$ |  |  | $\begin{array}{r} 8 \\ 2 \\ 12 \\ 3 \\ 9 \\ 10 \\ 5 \\ 4 \\ 7 \\ 10 \\ 10 \\ 9 \\ 8 \\ 16 \\ 18 \\ 24 \\ 4 \\ 6 \end{array}$ |  | . |  |
| $\mathrm{MDH}-3$ | aa $\times$ aa <br> $a b \times a b$ <br> $b b \times b b$ | $\begin{array}{r} 10 \\ 10 \\ 12 \\ 10 \\ 7 \\ 15 \\ 2 \\ 6 \\ 1 \\ 1 \\ 2 \\ 3 \\ 3 \\ 4 \\ 7 \\ 5 \end{array}$ | - | 4 14 7 7 $2:$ $7:$ $6:$ $6:$ 11 $19:$ 9 3 8 10 16 4 | . | - |  |  | . | . | . | . |  | ' |

${ }^{1}$ Heterozygous ab and homozygous bb progeny were impossible to distinguish, so they are summed under the bb column.

TABLE 7 cont.

| LOCUS | PARENTAL PHENOTYPES | aa | $a b$ | bb | ac | OFFSPRING PHENOTVPES |  |  |  |  | dd | an | bn | nn |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  | bc | cc | ad | bd | cd |  |  |  |  |
| MDH-5 | aa $\times$ aa | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  | - |  |  |  |  | . |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 24 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 15. |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 3 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  | - |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 8 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 16 |  |  |  |  |  |  |  |  |  |  | . |  |
|  |  | 18 |  |  |  |  |  |  |  |  |  |  |  |  |
|  | ac $X$ ac | 5 |  |  | 14 |  | 4 |  |  |  |  |  |  |  |
|  |  | 3 |  |  | 2 |  | 3 |  |  |  |  |  |  |  |
|  |  | 5 |  |  | 5 |  | 2 |  |  |  |  |  |  |  |
|  |  | 2 |  |  | 4 |  | 1 |  |  |  |  |  |  |  |
|  | ad X ad | 3 |  |  |  |  |  | 2 |  |  | 4 |  |  |  |
| MDH-6 | aa $X$ aa | 12 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 3 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 7 |  |  |  |  |  |  |  |  |  | - |  |  |
|  | - | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 7 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  | . |  |  |  |  |  |  |
|  |  | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 8 |  |  | . |  |  |  |  |  |  |  | . |  |
|  |  | 20 |  |  |  |  |  |  |  | . |  |  |  |  |
|  |  | 8 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 16 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 18 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  | - |  |  |  |  |  |  |  |  |  |  |
|  |  | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 24 |  |  |  |  |  |  |  |  |  |  | . |  |
|  |  | 15 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  | . |  |  |  |  | . |  |  |  |  |
|  | $a b \times a b$ | 5 | 4 |  | . |  |  |  |  |  |  |  |  |  |
| LAP-1 | bd $x$ bd |  |  | 3 |  | - |  |  | 4 |  | 1 |  |  |  |
|  | cd X ca' |  |  |  |  |  | 1 |  |  | 5 | 4 |  |  |  |
|  | dd $X$ dd |  |  |  |  |  |  |  |  |  | 9 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | 10 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | 12 |  |  |  |
|  |  |  |  |  |  |  | - |  |  |  | 7 |  |  |  |
|  |  |  | . |  |  |  |  |  |  |  | 10 |  |  |  |
|  |  |  |  |  |  | . |  |  |  |  | 16 |  |  |  |
|  |  |  |  |  |  | - |  |  |  |  | 18 |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  | 4 |  |  |  |
|  |  | , |  |  |  |  |  |  |  |  |  |  |  |  |

TABLE 7 cont.

| LOCUS | PARENTAL PHENOTYPES | aa | $a b$ | bb | ac | OFFSPRING PHENOTYPES |  |  |  |  | dd | an | bn | nn |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  | bc | cc | ad | bd | cd |  |  |  |  |
| LAP-2 | $b b \times b b$ |  |  | 7 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 10 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 12 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 8 |  |  |  |  |  | . |  |  |  |  |
|  |  |  |  | 10 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 16 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 18 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 4 |  |  |  |  |  |  |  |  |  |  |
|  | $b c \times b c$ |  |  | 4 |  | 5 |  |  |  |  |  |  |  |  |
|  | bn $X$ bn |  |  | 5 |  |  |  |  |  |  |  |  | 2 | 3 |
|  |  |  |  | 5 |  | - |  |  |  |  |  |  | 2 | 2 |
| DIA-1 | aa $X$ aa | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 16 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 11 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  | - |  |  |  |  |
|  |  | 18 |  |  |  | - |  |  |  |  |  |  |  |  |
|  |  | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  | $a b \times a b$ | 3 | 5 | 2 |  |  |  |  |  |  |  |  |  |  |
|  |  | 5 | 5 |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  | $6^{1}$ |  |  |  |  |  |  |  |  |  |  |
|  |  | 2 |  | $7{ }^{1}$ |  |  |  |  |  |  |  |  |  |  |
|  |  | 2 |  | $9^{\prime}$ |  |  |  |  |  |  |  |  |  |  |
|  |  | 4 |  | $4^{1}$ |  | . |  |  |  |  |  |  |  |  |
|  | $b b \times b b$ |  |  | 4 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 7 |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  | 6 |  |  |  |  |  |  | . |  |  |  |
| DIA-2 | aa $x$ aa | 16 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 11 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 7 |  |  |  |  |  |  |  | . |  |  |  |  |
|  |  | 18 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 11 |  |  |  |  |  | , |  |  |  |  |  |  |
|  |  | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 21 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 6 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 9 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 10 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | 8 |  | . |  |  | . |  |  |  |  |  |  |  |
|  |  | 4 |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

[^2] summed under the bb column.

### 3.4.1 Phosphoglucose Isomerase

PGI isozymes migrated to two regions of the gel (Figures 57). The anodal region was monomorphic while the slower zone was highly polymorphic. Two loci probably control the lower region since any pattern observed (including both band positions and intensities) could be explained with four alleles. A total of 14 alleles was invoked for these two loci, including a null for each. The loci share their most common allele so that a single heavy band was frequently seen in this region. It is unlikely that a third gene nearly always sharing the same allele is involved despite the taxa being hexaploid because different band intensity patterns would be expected, and because the occurrence of a genotype lacking that allele would be vanishingly small although several were actually observed. Determining which alleles belonged to which locus was a little more difficult because of the shared allele, but genotypes possessing two copies of an uncommon allele were assumed to be homozygous at one locus, allowing unambiguous assignment of the other alleles to the second locus. All alleles were assigned to the loci by finding genotypes homozygous for an uncommon allele at one locus and heterozygous at the other.

The anodal region consisted of three evenly spaced bands of which the slowest was much lighter than the other two. Although two loci fixed for different alleles would produce a threebanded pattern, the slowest band would not be lightest unless differential staining was involved. Because band intensity seems well correlated with allele dosage in PGI a three locus


Figure 5. Alleles of the PGI loci.

Figure 6. Genotypes of common PGI band patterns.


Figure 7. Photograph of PGI band patterns. Samples 1, 3 and 4 are 1aa2aa3aa4cc5cc, samples 2, 11, 12 and 13 are
1aa2aa3aa4dd5cc, samples 5, 6 and 10 are laa2aa3aa4df5cc, and samples 7, 8 and 9 are 1aa2aa3aafff5cc.
explanation is preferable. Two loci fixed for one allele and a third for the other would produce a 4:4:1 ratio of band intensities which fits the observed pattern.

PGI behaved as a dimeric enzyme in Bidens. This has also been reported in Festuca (Adams and Allard, 1977), wheat (Hart, 1979), Lolium (Nielsen, 1980), Clarkia (Weeden and Gottlieb, 1979), Gaura (Gottlieb and Pilz, 1977), Citrus (Torres et al. 1978), pitch pine (Guries and Ledig, 1978), ponderosa pine (Mitton et al., 1979), Douglas fir (El Kassaby et al. 1982) and Plectritis (Layton, 1980).

### 3.4.2 Phosphoglucomutase

Two independent zones of activity also appeared when gels were stained for PGM. Both were polymorphic and both best explained with two loci (Figures 8-10). The loci controlling the anodal region each had 5 alleles and the others had 3 and 4 alleles. Ail loci had a null allele.

PGM behaved like a monomeric enzyme, with heterozygotes at one locus having two bands instead of one. This substructure has also been reported in. Citrus (Torres et al., 1978), ponderosa pine (Mitton et al., 1979), Douglas fir (El Kassaby, 1982) and Plectritis (Layton, 1980).


Figure 8. Alleles of the PGM loci.

| 1 bb | 1 bb | 1 ab |
| :--- | :--- | :--- |
| 2 bb | 2 bb | 2 bc |
| 3 bb | 3 bb | 3 ab |
| 4 aa | 4 ab | 4 aa |

Figure 9. Genotypes of common PGM band patterns.


Figure 10. Photograph of PGM band patterns. Sample 1 is 3ab4aa, samples 2 and 3 are $3 b b 4 a b$, samples $4,7,8$ and 9 are $3 b b 4 a a$, and samples 5 and 6 are $3 b b 4 b b$. The faster zone is overstained and not decipherable.

### 3.4.3 Malate Dehydrogenase

MDH behaved like a dimeric molecule, had the most complex patterns and was controlled by more loci than any other enzyme system (Figures 11-13). The slowest region on the gels was not scored because of poor resolution in many taxa. The fastest band was identical in all individuals and probably controlled by one homozygous locus. The central area was variable, having from 4 to 10 bands, but certain sets of bands behaved independently of each other. The most common patterns are shown in Fig. 12. The zone labelled "C" appears to be controlled by two loci since variant 3-banded patterns have either light:heavy:light or heavy:medium:light band intensities reminiscent of $P G I$ and impossible to obtain with just one locus. Zone "B" is probably controlled by three loci with the simplest, heavy:heavy:light pattern representing two loci fixed. for one allele and the third for another. A third, intermediate allele at one of the faster loci would create the common 5-banded pattern and other faster alleles would cause the heavy:medium:light and light:heavy:light patterns at the anodal end of this zone. As before, two loci are needed to explain these intensity patterns at the top, and the third locus is necessary to account for the 3-or 5-banded patterns in the lower half of this zone.

One problem was assigning genotypes to the 5-banded pattern. The 3 - or 5 -banded patterns were often fixed for a population, in which case homozygosity for the relevant genes was inferred. In populations variable at this locus, however,


MDH-1 MDH-2 MDH-3 MDH-4 MDH-5 MDH-6

Figure 11. Alleles of the MDH loci.


Figure 12. Genotypes of common MDH band patterns.


Figure 13. Photograph of MDH band patterns. Samples 1 and 9 are indecipherable, samples 2, 3 and 5 are 2dd3aa4aa5ad6aa, samples 4 and 8 are 2dd3aa4aa5dd6aa, and samples 6 and 7 are 2dd3aa4aa5aa6aa. The most anodal band is not included in the photograph.
it was sometimes impossible to distinguish between heterozygotes and homozygotes for the 5-banded pattern. This is an unusual situation in electrophoresis, where allele dosage is usually revealed in simple relationships of band intensities, but in this instance patterns were so complex and expected intensity relationships sufficiently similar that unambiguous interpretation was not possible. One possible solution to this sort of situation is to calculate $F$, Wright's coefficient of inbreeding (which is theoretically the same for all loci) for a few other loci, and use this estimate to calculate the proportion of heterozygotes and homozygotes in the dominant 5banded phenotype. The $F$ values calculated for other polymorphic loci turned out to be so variable that using an average seemed quite arbitrary. Instead, the simpler Hardy-Weinberg model was used. The resulting gene frequencies for populations variable at this locus may not be strictly accurate, but they still afford a basis for comparison of populations.

MDH has also been reported to be dimeric in maize (Goodman et al., 1979), Acetabularia (Serov et al., 1979), Eucalyptus (Brown et al., 1975), Plectritis (Layton, 1980), pitch pine (Guries and Ledig, 1978), ponderosa pine (O'Malley et al., 1979), Douglas fir (El Kassaby, 1982) and lodgepole pine (Yeh and Layton, 1979).

### 3.4.4 Leucine Aminopeptidase

LAP gels had from one to four bands and seemed to be controlled by two loci, each with five active alleles and one null (figures 14-16). One of the alleles could not be unambiguously assigned to $a$ locus so the choice was made arbitrarily, but the effect of this is minor since the frequency of the allele was only . 01 . It behaved as a monomer, as has been reported for Picea abies (Lundkvist, 1974), Pinus sylvestris (Rudin, 1977), Pisum sativum (Scandalios and Espiritu, 1969) and Phaseolus (Wall, 1968).

### 3.4.5 Diaphorase

Diaphorase isozymes also formed complex patterns but the slowest bands seemed indeperdent of variation elsewhere. Two loci, one with four and the other with two alleles, were invoked to explain the patterns (Figures 17-18). Diaphorase behaved like a monomeric enzyme with heterozygotes having two bands.

### 3.5 American Taxa

The American taxa had very different banding patterns for many enzyme systems. There is no satisfactory way to establish gene homologies between the Hawaiian and American taxa by genetic analysis because the two groups will not hybridize.
$a-$
$b-$

e -
LAP-1 LAP-2

| ldd | 1 dd | 1 cd |
| :--- | :--- | :--- |
| 2 bb | 2 bc | 2 bb |

Figure 14. Alleles of the LAP Figure 15. Genotypes of common
loci.

LAP band patterns.


Figure 16. Photograph of LAP band patterns. Samples $1-7$ and 10 are $1 d d 2 b b$, and samples 8 and 9 are $1 d d 2 b c$.


Figure 17. Alleles of the DIA Figure 18. Genotypes of common loci.

DIA band patterns.

Inferences from band positions are the best method available and this was facilitated by the absence of variability within populations of the American taxa and by their bands being at either identical positions to those in the Hawaiian species or in radically different positions. All populations of American species were monomorphic, so allele frequencies of 1.00 were assigned either for alleles shared with Hawaiian plants or for alleles unique to the American species. The American taxa appear to have fewer loci than the Hawaiian taxa for some enzyme systems, presumably because they are not all hexaploid. Bidens frondosa is tetraploid and populations of B. tripartita are either tetraploid or hexaploid (Fedorov, 1974). Chromosome counts for B. amplissima and B. cynapifolia have not been reported. To compare taxa of different ploidy levels, missing loci were treated as being fixed for null alleles.

## IV. VARIABILITY WITHIN POPULATIONS

### 4.1 Hawaiian Populations

Sample sizes for many of the localities listed in Table 1 are too small for reasonable estimates of variability. Theoretically, a sample of 30 individuals provides a 0.95 probability of detecting an allele present in the population at a frequency of 0.05 . Because of the small size of many natural populations, however, samples of over 20 are treated as large enough to be representative. Bidens populifolia is included despite a sample of only 18 cuttings because they represent most of the individuals encountered at the site.

Table 8 lists several genetic measures of variability for 22 populations. Two values are given for percent polymorphic loci, number of alleles per polymorphic locus and number of alleles per locus: they were calculated using alleles present at a minimum frequency of either 0.05 or 0.01 . The values obtained using the 0.01 criterion may be more representative of large populations, but the others may provide a better basis for comparison of small and large samples. The polymorphic index value is identical to the mean or expected heterozygosity used by some authors and represents the heterozygote frequency (averaged over all loci) in a population conforming to HardyWeinberg assumptions. Alternatively, it can be considered as the proportion of loci at which the average individual is

TABLE 8. GENETIC MEASURES OF VARIABILITY IN 22 HAWAIIAN BIDENS POPULATIONS.

| TAXON AND POPULATION |  | n | ```% LDCI POLYMORPHIC'``` | NUMBER OF ALLELES PER POLYMORPHIC LOCUS ${ }^{2}$ | NUMBER OF ALLELES PER LOCUS? | $\begin{aligned} & \text { POLYMORPHIC } \\ & \text { INDEX } \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  | - |  |  |
| ASYM | B4 | 27 | 30.4 (30.4) | 3.57 (3.14) | .1.48 (1.35) | 0.118 |
| CERV | B87 | 38 | 34.8 (17.4) | 3.25 (4.75) | 1.52 (1.22) | 0.073 |
| FORB F | F B14 | 25 | 34.8 (26.1) | 3.25 (3.67) | 1.52 (1.35) | 0. 102 |
| HAWA | B50 | 52 | 34.8 (26.1) | 3.88 (4.00) | 1.74 (1.43) | 0. 135 |
|  | B231 | 137 | 30.4 (26.1) | 3.86 (3.83) | 1.56 (1.39) | 0.099 |
| MENZ | F B109 | 48 | 47.8 (43.5) | 2.91 (2.30) | 1.78 (1.39) | 0.129 |
|  | B130 | 57 | 47.8 (39.1) | 2.91 (2.67) | 1.78 (1.43) | O. 148 |
|  | B2 18 | 100 | 43.5 (30.4) | 3.30 (3.43) | 1.83 (1.43) | 0.115 |
|  | B219 | 61 | 34.8 (30.4) | 2.88 (3.14) | 1.39 (1.35) | 0.103 |
| MICR M | M B78 | 21 | 43.5 (39.1) | 2.70 (2.78) | 1.56 (1.48) | 0. 125 |
| MICR C | C B149 | 80 | 52.2 (39.1) | 2.67 (2.56) | 1.78 (1.39) | 0. 136 |
| MICR K | - B197 | 22 | 43.5 (43.5) | 2.70 (2.60) | 1.56 (1.52) | 0.144 |
| MOLO | B72 | 62 | 30.4 (17.4) | 3.00 (4.25) | 1.30 (1.13) | 0.043 |
| POPU | B42 | 18 | 30.4 (26.1) | 3.00 (3.33) | 1.30 (1.26) | 0.084 |
| SAND | $5 \mathrm{B4} 4$ | 21 | 26.1 (17.4) | 3.33 (4.50) | 1.26 (1.17) | 0.058 |
|  | B200 | 79 | 47.8 (34.8) | 3.00 (2.62) | 1.83 (1.30) | 0.091 |
| SAND $C$ | C B34 | 21 | 47.8 (43.5) | 2.45 (2.50) | 1.56 (1.48) | 0.116 |
| TORT | B15 | 79 | 34.8 (26.1) | 2.88 (3.33) | 1.39 (1.26) | 0.074 |
|  | B37 | 64 | 39.1 (34.8) | 3.67 (3.00) | 1.83 (1.43) | 0.082 |
|  | B2 13 | 110 | 43.5 (34.8) | 3.50 (2.75) | 1.91 (1.35) | 0. 103 |
|  | 8257 | 49 | 47.8 (39.1) | 3.55 (3.00) | 2.09 (1.57) | 0. 123 |
| WIEB | B259 | 35 | 39.1 (30.4) | 2.89 (3.00) | 1.52 (1.30) | 0.079 |
| MEAN |  |  | 39.4 (31.6) | 3.14 (3.23) | 1.61 (1.36) | 0. 104 |
| SD |  |  | 7.69 (8.29) | 0.40 (0.68) | 0.22 (0.11) | 0.028 |

1 Values are given for loci at which at which the most common allele has a frequency of $\leq 0.99$ or $\leq 0.95$ (in brackets).
${ }^{2}$ Values are given for alleles present at a frequency of $\geq 0.01$ or $\geq 0.05$ (in brackets).
heterozygous. It provides a useful relative measure of variability regardless of the actual proportion of heterozygotes in natural populations.

All of the Bidens populations fall well within the range of variability found for plant populations described in a recent review (Gottlieb, 1981). The average values for percent polymorphic loci, number of alleles per polymorphic locus and polymorphic index are much higher than the averages calculated for 28 selfing species (4.4, 2.26 and . 001 , respectively) and similar to or somewhat higher than for 21 outcrossing species (37, 2.9 and . 086, respectively). Populations of Hawaiian Bidens are therefore not exceptional, but tend to have higher levels of variability than most species examined to date.

Of the 23 loci surveyed in populations of Hawaiian Bidens, 14 (60.9\%) are polymorphic. Seventy-one alleles were found in all, for an average of 3.09 alleles per locus and 4.43 alleles per polymorphic locus for the group as a whole.

### 4.2 Hawaiian Taxa

In order to include information from plants at all localities, measures of variability were also calculated for all taxa in which the number of plants sampled was over 20 (again with the exception of $B$. populifolia, which fell just short of this criterion). These are listed in Table 9 along with means and standard deviations for the 15 taxa. Bidens mauiensis is

TABLE 9. GENETIC MEASURES OF VARIABILITY IN 15 HAWAIIAN BIDENS TAXA.

| SPECIES | n | \% LOCI <br> POLYMORPHIC | NUMBER OF ALLELES PER POLYMORPHIC LOCUS ${ }^{2}$ | Number of ALLELES PER LOCUS ${ }^{2}$ | POLYMORPHIC <br> INDEX |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  |  |
| ASYM | 38 | 39.1 (34.8) | 3.22 (3.25) | 1.65 (1.52) | O. 142 |
| CERV | 65 | 47.8 (39.1) | 3.09 (2.67) | 1.87 (1.43) | -0. 0.120 |
| FORB F | 38 | 47.8 (34.8) | 2.64 (2.88) | 1.65 (1.39) | 0.110 |
| HAWA | 199 | 39.1 ( 21.7 ) | 3.44 (4.40) | 1.74 (1.35) | O. 116 |
| MAUI | 26 | 56.5 (52.2) | 2.46 (2.50) | 1.78 (1.70) | O. 172 |
| MENZ F | 287 | 52.2 (39.1) | 3.33 (2.67) | 2.13 (1.43) | O. 140 |
| MICR M | 40 | 52.2 (43.5) | 2.67 (2.50) | 1.78 (1.48) | O. 137 |
| MICR C | 80 | 52.2 (39.1) | 2.67 (2.56) | 1.78 (1.39) | 0. 136 |
| MICR K | 23 | 43.5 (39.1) | 3.00 (2.89) | 1.70 (1.52) | 0.150 |
| MOLO | 64 | 30.4 (13.0) | 3.00 (5.67) | 1.30 (1.13) | 0.042 |
| POPU | 19 | 30.4 (26.1) | 3.00 (3.33) | 1.30 (1.26) | 0.082 |
| SAND S | 124 | 52.2 (39.1) | 3.00 (2.44) | 1.96 (1.35) | 0.099 |
| SAND C | 23 | 47.8 (43.5) | 2.64 (2.40) | 1.65 (1.43) | O. 117 |
| TORT | 329 | 60.9 (39.1) | 3.14 (2.56) | 2.30 (1.39) | 0.121 |
| WIEB | 35 | 39.1 (30.4) | 2.89 (3.00) | 1.52 (1.30) | 0.079 |
| MEAN |  | 46.1 (35.6) | 2.95 (3.05) | 1.74 (1.40) | 0.118 |
| SD |  | 8.99 (9.62) | 0.28 (0.89) | 0.27 (0.13) | 0.032 |

[^3]included in this analysis although it was excluded from the last one because of small sample sizes from each population.

In general, values are higher for taxa than for populations, showing that some differentiation of populations within species has occurred. The only measure of variability that is consistently lower for taxa is the number of alleles per polymorphic locus; this occurs because there is a relatively greater difference in the number of polymorphic loci in taxa than in the total number of alleles found in taxa.

### 4.3 American Species

In contrast to Hawaiian taxa, the weedy American species showed no variability in any population. No polymorphic loci exist in any of the populations, resulting in the minimum value of 1 allele per locus and a polymorphic index of 0 . For the American taxa as a group, 33 alleles exist at the nominal 23 loci for an average 1.43 alleles per locus and 2.25 alleles per polymorphic locus.

## V. DIFFERENTIATION OF POPULATIONS

### 5.1 Genetic Identities

Many indices of genetic similarity have been proposed for comparison of populations using allele frequency data (Sanghvi, 1953; Cavalli-Sforza and Edwards, 1967; Balakrishnan and Sanghvi, 1968; Hedrick, 1971; Rogers, 1972; Nei, 1972). Nei's genetic identity and genetic distance measures are preferable to the others, however, because they are the only ones that have a biological basis as opposed to being simply abstract measures: Nei's genetic distance (1972) estimates the average number of codon differences per locus that are detectable using electrophoresis. An even more compelling reason to use Nei's indices is that they are used to estimate similarity of populations in most other plant populations studies, facilitating direct comparison of results.

The formula for Nei's genetic identity is:

$$
I=\frac{J_{x y}}{\sqrt{J_{x} J_{y}}}
$$

where $J_{x y}, J_{x}$ and $J_{y}$ are the means of $\sum x_{i} y_{i}, \sum x_{i}{ }^{2}$ and $\sum y_{i}{ }^{2}$ over all loci, and $x$; and $y$; are the frequencies of the i-th allele in the two populations being compared. Genetic identity can range from 0 to 1 and is affected by both the presence and the
frequency of alleles. The value for a pair of populations is 1 if the populations not only share all of their alleles but also have them at identical frequencies. The value is 0 if the populations share no alleles at the loci studied. Nei's genetic distance is defined as:

$$
D=-\ln I
$$

which approximates $1-1$ at high values of $I$.
Gottlieb (1977) found average genetic identities for conspecific populations in 22 flowering plant species to be $0.95 \pm 0.02$. Thirteen comparisons of congeneric plant species had a mean genetic identity of $0.67 \pm 0.07$. In a more recent review considering only the studies in which 11 or more loci were surveyed (Gottlieb, 1981), the mean $I$ for conspecific populations is 0.975 for inbreeding species and 0.956 for outbreeding species, with standard errors of 0.01 and 0.11 respectively. Table 10 lists the genetic identity and distance values for all pairwise comparisons of Bidens populations. The values for comparisons involving only Hawaiian populations (including both intra- and intertaxon comparisons) range from 0.872 to 0.996 with a mean of 0.949 , while all comparisons of Hawaiian and American taxa range from 0.510 to 0.727 with a mean of 0.603 and a standard deviation of 0.054 . The similarities between all of the Hawaiian populations are what would be expected of intraspecific comparisons even though 14 taxa are involved, while their relationship to populations of American

TABLE 10. GENETIC IDENTITY AND DISTANCE VALUES' FOR POPULATIONS OF HAWAIIAN AND AMERICAN BIDENS TAXA.


[^4]table 10. cont.

| POPULATION |  |  | POPU | SAND | S | SAND | 5 | SAND C | TORT | TORT | TORT | TORT | WIEB | AMPL | TRIP | FRON | CYNA |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ASYM |  | B4 | -0.939 | 0.914 |  | 0.920 |  | 0.917 | 0.915 | 0.895 | 0.946 | 0.947 | 0.914 | 0.596 | 0.596 | 0.647 | 0.503 |
| CERV |  | 887 | 0.939 | 0.910 |  | 0.949 |  | 0.926 | 0.970 | 0.958 | 0.974 | 0.973 | 0.957 | 0.590 | 0.590 | 0.668 | 0.500 |
| FORB | F | B 1.1 | 0.949 | 0.924 |  | 0.943 |  | 0.927 | 0.953 | 0.941 | 0.973 | 0.967 | 0.952 | 0.595 | 0.595 | 0.621 | 0.503 |
| HAWA |  | B231 | 0.948 | 0.919 |  | 0.930 |  | 0.920 | 0.943 | 0.924 | 0.983 | 0.979 | 0.933 | 0.583 | 0.583 | $0.629^{\circ}$ | 0.491 |
| HAWA |  | B50 | 0.918 | 0.886 |  | 0.906 |  | 0.894 | 0.923 | 0.903 | 0.963 | 0.953 | 0.913 | 0.613 | 0.613 | 0.665 | 0.520 |
| MENZ | F | B109 | 0.957 | 0.955 |  | 0.980 |  | 0.958 | 0.990 | 0.975 | 0.971 | 0.972 | 0.981 | 0.579 | 0.579 | 0.669 | 0.486 |
| MENZ | F | E130 | 0.950 | 0.946 |  | 0.970 |  | 0.955 | 0.968 | 0.958 | 0.953 | 0.950 | 0.970 | 0.631 | 0.631 | 0.723 | 0.537 |
| MENZ | F | B2 18 | 0.964 | 0.963 |  | 0.984 |  | 0.971 | 0.968 | 0.960 | 0.947 | 0.943 | 0.981 | 0.634 | 0.634 | 0.727 | 0.542 |
| MENZ | F | B2 19 | 0.959 | 0.952 |  | 0.981 |  | 0.964 | 0.975 | 0.967 | 0.960 | 0.957 | 0.981 | 0.621 | 0.621 | 0.711 | 0.529 |
| MICR | M | 878 | 0.981 | 0.972 |  | 0.970 |  | 0.964 | 0.964 | 0.952 | 0.989 | 0.985 | 0.971 | 0.615 | 0.615 | 0.669 | 0.522 |
| MICR | C | 8149 | 0.933 | 0.946 |  | 0.949 |  | 0.940 | 0.930 | 0.917 | 0.912 | 0.911 | 0.941 | 0.620 | 0.620 | 0.708 | 0.527 |
| MICR | K | E 197 | 0.935 | 0.922 |  | 0.953 |  | 0.934 | 0.974 | 0.965 | 0.972 | 0.973 | 0.962 | 0.601 | 0.601 | 0.667 | 0.507 |
| MOLO |  | B72 | 0.975 | 0.977 |  | 0.983 |  | 0.979 | 0.946 | 0.941 | 0.928 | 0.930 | 0.971 | 0.602 | 0.602 | 0.687 | 0.513 |
| POPU |  | B42 |  | 0.986 |  | 0.979 |  | 0.980 | 0.952 | 0.944 | 0.967 | 0.964 | 0.972 | 0.619 | 0.619 | 0.675 | 0.528 |
| SAND | S | B4.4 | 0.014 |  |  | 0.975 |  | 0.980 | 0.941 | 0.934 | 0.945 | 0.942 | 0.966 | 0.601 | 0.601 | 0.669 | 0.512 |
| SAND | 5 | B200 | 0.021 | 0.025 |  |  |  | 0.983 | 0.981 | 0.975 | 0.965 | 0.965 | 0.992 | 0.606 | 0.606 | 0.693 | 0.515 |
| SAND | C | B34 | 0.020 | 0.020 |  | 0.017 |  |  | 0.955 | 0.962 | 0.950 | 0.951 | 0.977 | 0.608 | 0.608 | 0.685 | 0.515 |
| TORT |  | B37 | 0.049 | 0.061 |  | 0.020 |  | 0.046 |  | 0.990 | 0.980 | 0.981 | 0.987 | 0.580 | 0.580 | 0.669 | 0.490 |
| TORT |  | B15 | 0.058 | 0.069 |  | 0.025 |  | 0.039 | 0.010 |  | 0.967 | 0.973 | 0.983 | 0.576 | 0.576 | 0.667 | 0.486 |
| TORT |  | B213 | 0.034 | 0.057 |  | 0.036 |  | 0.051 | 0.021 | 0.034 |  | 0.996 | 0.972 | 0.598 | 0.598 | 0.656 | 0.506 |
| TORT |  | B257 | 0.036 | 0.060 |  | 0.035 |  | 0.050 | 0.019 | 0.028 | 0.004 |  | 0.970 | 0.589 | 0.589 | 0.650 | 0.496 |
| WIEB |  | B260 | 0.028 | 0.035 |  | 0.008 |  | 0.024 | 0.013 | 0.018 | 0.028 | 0.030 |  | 0.598 | 0.598 | 0.683 | 0.508 |
| AMPL |  |  | 0.479 | 0.508 |  | 0.500 |  | 0.498 | 0.544 | 0.551. | 0.515 | 0.530 | 0.514 |  | 0.957 | 0.826 | 0.739 |
| TRIP |  |  | 0.479 | 0.508 |  | 0.500 |  | 0.498 | 0.544 | 0.551 | 0.515 | 0.530 | 0.514 | 0.044 |  | 0.870 | 0.739 |
| FRON |  |  | 0.393 | 0.403 |  | 0.367 |  | 0.378 | 0.402 | 0.405 | 0.421 | 0.431 | 0.381 | 0.191 | 0.140 |  | 0.652 |
| CYNA |  |  | 0.638 | 0.670 |  | 0.663 |  | 0.663 | 0.714 | 0.721 | 0.681 | 0.702 | 0.678 | 0.302 | 0.302 | 0.427 |  |

taxa conform to expected values for interspecific comparisons. Despite the high mean and small range of genetic identities, the comparisons of Hawaiian Bidens populations can be analyzed according to taxonomic relationship. Table 11 lists the mean and associated values for intrataxon, intersubspecific and interspecific comparisons. A one-way analysis of variance shows these "treatments" to have a significant effect on sample means $(F=12.61, P<0.001, d . f .=21$ using the number of populations minus one as the minimum estimate of degrees of freedom). Comparison of sample means using a 1-tailed t-test with the error mean sum of squares for variance (Sokal and Rohlf, 1969) gives a significant value of 2.12 for intrataxon vs. intersubspecific comparisons (minimum d.f. $=16-1=15$ ) but a nonsignificant value of 0.601 for intersubspecific vs. interspecific comparisons $(\mathrm{df}=21, \mathrm{P}<0.28)$. The genetic identities of populations of the same taxon are thus significantly higher than those of populations from different taxa, but populations of different subspecies of one species are no more similar than populations of different species.

The genetic identity values can also be analyzed according to the extent of geographic separation of populations to determine if their affinities are related to the distance between them. The treatments for this ANOVA are comparisons of populations from the same island, from adjacent islands and from distant islands. All intrataxon comparisons were excluded because populations of the same tax had already been shown to be significantly more similar than populations of different

TABLE 11. GENETIC IDENTITIES FOR TAXONOMIC COMPARISONS OF 22 HAWAIIAN BIDENS POPULATIONS.

| COMPARISON | $n$ | HIGH | LOW | MEAN | SD |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| INTRATAXON | 14 | 0.996 | 0.967 | 0.9814 | 0.0085 |
| INTERSUBSPECIES | 5 | 0.983 | 0.919 | 0.9548 | 0.0285 |
| INTERSPECIES | 212 | 0.992 | 0.872 | 0.9472 | 0.0254 |

TABLE 12. GENETIC IDENTITIES FOR INTERTAXON GEOGRAPHIC COMPARISONS OF 22 HAWAIIAN BIDENS POPULATIONS.

|  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
| COMPARISON | $n$ | HIGH |  |  |

taxa. Intrataxon comparisons would have biased the analysis because populations of the same taxon are usually found on the same island or on adjacent islands. Maui, Molokai and Lanai were treated as one island because of their proximity and the existence of land bridges between them during the pleistocene (Stearns, 1966). Table 12 lists the range, mean and standard deviation for each "treatment". A one-way ANOVA again gives a significant value $(F=3.84, \mathrm{P}<0.05, \mathrm{df}=2,21$ ). A priori 2-tailed t-tests based on the ANOVA show a significant difference between genetic identities of comparisons between populations on the same island and on adjacent islands ( $t=2.56, \mathrm{df}=21, \mathrm{P}<0.05$ ) and between genetic identities of populations from adjacent and distant islands $(t=2.08, d f=21, P=0.05)$. Surprisingly, the greatest similarities are found between populations of different taxa on adjacent islands, with populations of different taxa on the same island being least similar.

Table 13 lists genetic identities and distances for Bidens taxa. All individuals sampled are included in this analysis. The overall results are similar to those obtained from comparisons of individual populations, but this analysis includes $B$. mauiensis.

TABLE 13. GENETIC IDENTITY AND DISTANCE VALUES' FOR HAWAIIAN AND AMERICAN BIDENS TAXA.

${ }^{1}$ Genetic identity values are in the upper region of the table, and genetic distances in the lower region.

TABLE 13. cont.

| TAXON | POPU | SAND S | SAND C | TORT | WIEB | $\triangle M P L$ | TRIP | FRON | CYNA |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| ASYM | 0.961 | 0.954 | 0.950 | 0.954 | 0.936 | 0.607 | 0.607 | 0.666 | 0.513 |
| CERV | 0.955 | 0.960 | 0.948 | 0.991 | 0.965 | 0.600 | 0.600 | 0.668 | 0.507 |
| FORB F | 0.969 . | 0.968 | 0.953 | 0.981 | 0.965 | 0.610 | 0.610 | 0.645 | 0.518 |
| HAWA | 0.942 | 0.935 | 0.921 | 0.973 | 0.930 | 0.591 | 0.591 | 0.639 | 0.499 |
| MAUI | 0.969 | 0.957 | 0.959 | 0.962 | 0.935 | 0.596 | 0.596 | 0.640 | 0.501 |
| MENZ F | 0.966 | 0.984 | 0.974 | 0.972 | 0.983 | 0.625 | 0.625 | 0.715 | 0.531 |
| MICR M | 0.972 | 0.974 | 0.959 | 0.983 | 0.962 | 0.601 | 0.601 | 0.653 | 0.507 |
| MICR C | 0.932 | 0.951 | 0.941 | 0.920 | 0.938 | 0.620 | 0.620 | 0.708 | 0.527 |
| MICR K | 0.935 | 0.954 | 0.941 | 0.980 | 0.963 | 0.601 | 0.601 | 0.666 | 0.506 |
| MOLO | 0.976 | 0.984 | 0.979 | 0.937 | 0.966 | 0.603 | 0.603 | 0.688 | 0.514 |
| POPU |  | 0.988 | 0.981 | 0.966 | 0.966 | 0.619 | 0.619 | 0.675 | 0.529 |
| SAND S | 0.012 |  | 0.990 | 0.975 | 0.987 | 0.609 | 0.609 | 0.686 | 0.517 |
| SAND C | 0.020 | 0.010 |  | 0.964 | 0.976 | 0.605 | 0.605 | 0.684 | 0.512 |
| TORT | 0.035 | 0.025 | 0.037 |  | 0.978 | 0.596 | 0.596 | -0.662 | 0.503 |
| WIEB | 0.034 | 0.013 | 0.024 | 0.022 |  | 0.597 | 0.597 | 0.683 | 0.506 |
| AMPL | 0.479 | 0.496 | 0.503 | 0.518 | 0.516 |  | 0.957 | 0.826 | 0.739 |
| TRIP | 0.479 | 0.496 | 0.503 | 0.518 | 0.516 | 0.044 |  | 0.870 | 0.739 |
| FRON | 0.394 | 0.376 | 0.380 | 0.412 | 0.381 | 0.191 | 0.140 |  | 0.652 |
| CYNA | 0.638 | 0.659 | 0.669 | 0.688 | 0.681 | 0.302 | 0.302 | 0.427 |  |

### 5.2 Gene Diversity

The organization of genetic variability within a group of organisms can be investigated using gene diversity analysis (Nei, 1975). This method reveals the subdivision of genetic variability in Hawaiian Bidens as a whole instead of simply making pairwise comparisons of similarity. Total gene diversity is subdivided into components using:

$$
\mathrm{H}_{T}=\mathrm{H}_{S}+\mathrm{D}_{S T},
$$

where $H_{T}$ is the total gene diversity, $H_{S}$ is the average gene diversity within populations and $D_{\rho T}$ is the average gene diversity among populations. Values of $H_{T}$ and $H_{s}$ are obtained independently for each locus and then averaged for all loci. $H_{T}$ is calculated as

$$
H=1-\sum \bar{x}_{i}{ }^{2},
$$

where $\bar{x}_{i}$ is the mean frequency of the i-th allele over all populations. $H_{s}$ is calculated for each population by

$$
H_{S}=1-\sum x_{i}{ }^{2},
$$

(which is equivalent to expected heterozygosity in a population using the Hardy-Weinberg model and is identical to the polymorphic index), and is then averaged for all populations. Dst is obtained by subtraction.

If each population maintains the amount of genetic variability found in the group as a whole, then $H_{T}$ and $H_{s}$ will
be identical and $D_{s T}$ will equal 0. If populations differ in allele frequencies (whether because of mutation, gene flow, random drift, selection or meiotic drive), $H_{s}$ will be smaller than $H_{T}$. The relative extent of differentiation among populations is given by

$$
\mathrm{G}_{S T}=\mathrm{D}_{S T} / \mathrm{H}_{T},
$$

where $G_{s T}$ is the coefficient of gene differentiation and can vary from 0 to 1. Alternatively, $D_{S T} / H_{s}$ is used by some authors (e.g., Brown, 1979).

Table 14 presents gene diversity values separately for the 14 polymorphic loci in populations of Hawaiian Bidens. Considerable variability among loci is evident even without including the monomorphic loci, all of which have values of 0 for each category. No clear pattern seems to exist, and the conclusion is that many loci should be sampled in order not to bias the results of a study of genetic variability.

Table 15 gives the results of gene diversity analysis at all loci for all populations, populations within a taxon and for subspecies within a species. The amount of differentiation among populations, as measured by $G_{s T}$, is greatest when all populations are considered, is lower for different subspecies within a species, and is least for populations belonging to the same taxon. This pattern supports the observation that genetic identities are highest when populations of the same tax are compared.

When only polymorphic loci are considered, $\mathrm{D}_{\text {ST }} / \mathrm{H}_{5}$ averages 0.114 for populations of one taxon and is 0.419 for all

TABLE 14. GENE DIVERSITY AT 14 POLYMORPHIC LOCI IN 22 POPULATIONS OF HAWAIIAN BIDENS.

|  |  |  |  |  |  |
| :--- | :---: | :---: | :---: | :---: | :---: |
| LOCUS | $H_{\mathbf{T}}$ | $H_{\mathbf{S}}$ | $D_{\mathbf{S T}}$ | $G_{\mathbf{S T}}{ }^{1}$ | $D_{\mathbf{S T}} / H_{\mathbf{S}}$ |
|  |  |  |  |  |  |
|  |  |  |  |  |  |
| PGI-4 | 0.4089 | 0.3086 | 0.1003 | 0.25 | 0.32 |
| PGI-5 | 0.2943 | 0.2428 | 0.0515 | 0.18 | 0.21 |
| PGM-1 | 0.2745 | 0.1810 | 0.0935 | 0.34 | 0.52 |
| PGM-2 | 0.0988 | 0.0867 | 0.0121 | 0.12 | 0.14 |
| PGM-3 | 0.2101 | 0.1727 | 0.0374 | 0.18 | 0.22 |
| PGM-4 | 0.3487 | 0.2948 | 0.0540 | 0.15 | 0.18 |
| LAP-1 | 0.1467 | 0.1233 | 0.0234 | 0.16 | 0.19 |
| LAP-2 | 0.3478 | 0.2508 | 0.0970 | 0.28 | 0.39 |
| DIA-1 | 0.5320 | 0.28 .77 | 0.2443 | 0.46 | 0.85 |
| DIA-2 | 0.0667 | 0.0435 | 0.0232 | 0.35 | 0.53 |
| MDH-2 | 0.0612 | 0.0500 | 0.0112 | 0.18 | 0.22 |
| MDH-3 | 0.4505 | 0.2310 | 0.2195 | 0.49 | 0.95 |
| MDH-5 | 0.1306 | 0.1012 | 0.0293 | 0.22 | 0.29 |
| MDH-6 | 0.0072 | 0.0068 | 0.0004 | 0.06 | 0.06 |
|  |  |  |  |  |  |

${ }^{1} G_{S T}=D_{S T} / H_{T}$.

TARLE 15. GENE DIVERSITY FOR ALL LOCI IN HAWAIIAN BIDENS.

| CATEGORY | $n$ | $H_{T}$ | $H_{S}$ | $D_{S T}$ | $G_{S T}$ | $D_{S T} / H_{S}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| A11 populations | 22 | 0.147 | 0.104 | 0.043 | 0.295 | 0.419 |

Populations of a taxon

| HAWA |  | 2 | 0.125 | 0.118 | 0.007 | 0.056 | 0.059 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| MENZ F | 4 | 0.136 | 0.124 | 0.012 | 0.088 | 0.097 |  |
| SAND S |  | 2 | 0.086 | 0.074 | 0.012 | 0.140 | 0.162 |
| TORT |  | 4 | 0.108 | 0.095 | 0.013 | 0.120 | 0.137 |
| MEAN |  |  | 0.114 | 0.103 | 0.011 | 0.101 | 0.114 |

Subspecies
MICR
3
0.171
0.135
0.036
0.211
0.267
${ }^{1} \mathrm{G}_{S T}=\mathrm{D}_{S T} / H_{T}$.
populations. Brown (1979) calculated averages of 0.17 and 1.18 for 20 outbreeding and 13 inbreeding species, respectively. All of the values for Hawaiian Bidens are low considering that species with presumably mixed mating systems are involved. Again, this is in agreement with the unusually high genetic identities found among all populations of this group.

### 5.3 Principal Component Analysis

The relationships of Bidens populations and taxa can be illustrated using principal component analysis (PCA). This technique ordinates the groups being studied in a space of fewer dimensions than the number of variables measured by finding correlations between the variables. Unlike clustering techniques which force the groups into a hierarchic classification, PCA permits overlapping clusters and is preferable in situations where obvious clusters are absent (Sokal, 1974). The axes of variation calculated in the analysis are linear combinations of the original variables and account for the largest possible proportion of variation. Each successive axis thus accounts for a progressively smaller amount of the total variability present, and each may be made up of components of all of the original variables.

Figures 19-21 are plots of the 22 Hawaiian Bidens populations on the first and second, first and third, and second and third PCA axes, respectively, of analyses where allele


AXIS 1 ( $13.4 \%$ variance)

Figure 19. Ordination of 22 Hawaiian Bidens populations on the first and second PCA axes.


Figure 20. Ordination of 22 Hawaiian Bidens populations on the first and third PCA axes.


Figure 21. Ordination of 22 Hawaiian Bidens populations on the second and third PCA axes.
frequencies are the variables describing each population. The most notable conclusion from the analysis, aside from the fact that only one population seems consistently disjunct from the rest, is that the first axis accounts for only $13.4 \%$ of the variance present, and the first seven account for a mere $64.3 \%$. The inability of this method to find an axis explaining a larger proportion of the total variability demonstrates the hyperspherical nature of the data set and emphasizes the absence of pattern in the variation of the populations. Figure 22 is an ordination of 15 Hawaiian Bidens taxa on the first two PCA axes and shows essentially the same thing, with no taxon appearing disjuct from the rest.

When American taxa are included (Figures 23 and 24) two non-overlapping clusters appear; one for the American and one for the Hawaiian plants. The first seven axes still account for only $63.2 \%$ (when populations were used) and $69.0 \%$ (when taxa were used) of the total variance, however.

The coefficients describing the contribution of variables to each axis are also low. Their absolute values for the first axis of the analysis of 22 Hawaiian populations, for example, range from 0.008 to only 0.257 and no single allele has a predominant effect.


Figure 22. Ordination of 15 Hawaiian Bidens taxa on the first and second PCA axes.


```
AXIS 1 (22.4\% variance)
```

Figure 23. Ordination of 22 Hawaiian Bidens populations and 4 populations of American taxa on the first and second PCA axes.


Figure 24. Ordination of 15 Hawaiian and 4 American Bidens taxa on the first and second PCA axes.

### 5.4 Cluster Analysis

The Hawaiian populations were also subjected to a linkage clustering technique producing a dendrogram. An unweighted pair-group method using arithmetic averages (Sneath and Sokal, 1973) results in the dendrogram of Figure 25. Although dendrograms produce clear arrangements of populations belonging to well-differentiated species in Plectritis (Layton, 1980) and Limnanthes (Ritland, pers. comm.), the results with Hawaiian Bidens are less satisfactory. Populations belonging to a single species or subspecies seem to be separated as often as not and the groups formed by the analysis are therefore probably artifacts of the method rather than a reflection of any actual hierarchical relationship existing among them. The fact that most clusters are formed simply by the addition of one population also suggests that structure is being forced upon the data rather than being revealed. The analysis demonstrates yet again the close similarities of the Hawaiian plants.


Figure 25. Dendrogram of 22 Hawaiian Bidens populations.

## VI. DISCUSSION

Most enzyme systems or subsets of an enzyme system showing independent variation in Hawaiian Bidens appear to be controlled by two rather than three loci. This is unexpected in a plant known to be hexaploid. Gene duplication events can occur through unequal crossing-over (Ohno, 1970), by crosses between individuals with different, partially overlapping, reciprocal translocations (Burnham, 1962), or by transposition (Dover, 1982), but these are mechanisms affecting only one or a few loci. A duplicated PGI locus in Clarkia (Gottlieb and Weeden, 1979) and duplicated MDH and ADH loci in maize (Goodman et al., 1980; Schwartz and Endo, 1966) are examples of these phenomena. The gene duplication observed in Bidens, however, is a result of genome duplication affecting all loci. Since enzyme systems assayed with natural substrates have similar numbers of loci governing their production in diploid flowering plants (Gottlieb, 1982), three times as many loci should be identified in a hexaploid. Nearly all homoeologous loci are expressed in hexaploid Triticum species (Hart and Langston, 1977) although the duplicated genes have diverged in structure and function. The genetic control of all enzyme systems studied in tetraploid Tragopogon species can be accounted for by the genes inherited from their diploid progenitors, and even the relative activities of gene products at $A D H$ loci in the diploids are retained in the tetraploids (Roose and Gottlieb, 1976).

The loss of duplicate gene expression in Hawaiian Bidens has presumably occurred by the fixation of null alleles at
certain loci. The silencing of a locus is facilitated in polyploids by the presence of other loci capable of fulfilling the biochemical requirements of organisms. Ferris and Whitt (1980) found that tetraploid catostomid fishes retain duplicated gene expression at only $47 \%$ of their isozyme loci. They suggest that the rate of formation and fixation of null alleles may be characteristic of some enzyme systems because they found singly expressed loci to be more polymorphic than duplicate loci. A large number of alleles would be expected to arise at a locus at which mutations easily alter the enzyme produced: many of these would specify molecules differing only in electrophoretic mobility, but some would specify molecules sufficiently different that enzyme activity is no longer apparent (null alleles). Because null alleles seem to exist at low frequencies at several isozyme loci studied in Hawaiian Bidens, it is not unlikely that they have arisen and become fixed at other loci as well.

The relatively high levels of genetic polymorphism within populations of Hawaiian Bidens compared with other higher plants are in agreement with observations of hexaploid Triticum, in which extensive differentiation among genomes has occurred (Jaaska, 1969; Hart and Langston, 1977). The unexpectedly low levels of differentiation of the Hawaiian populations at isozyme loci cannot therefore be attributed to lack of variability, as it can in the genetically depauperate Sullivantia species (Soltis, 1982). Although a large number of alleles exist at many of the loci, very little correlated differentiation is
observed at the isozyme loci as a whole. The genetic identities show that differentiation among the Hawaiian taxa is no greater than the degree of differentiation that has been documented for populations of the same taxon in continental species. Fourteen different classifications would result if a taxonomy were erected on the basis of each polymorphic locus, and the populations could be lumped into one species on the basis of the high genetic identity values if all loci were considered together.

Patterns exist in the genetic identity data despite the generally high values obtained for all possible comparisons of Hawaiian Bidens populations. The significantly different genetic identities of intrataxon and intertaxon comparisons show that populations of the same species are more similar to each other than to populations of other species. The gene diversity analysis shows greatest differentiation among populations when all Hawaiian populations are considered and least differentiation when only the populations belonging to one tax are considered, corroborating the existence of a taxonomic pattern. The PCA and dendrogram analyses fail to reveal groups of populations correlated with taxonomic classification based on morphology, so no correlation between morphological characters and isozyme characters exists above the level of populations of the same taxon. On morphological grounds, Bidens molokaiensis and $\underline{B}$. maviensis are very similar, and $\underline{B}$. forbesii and $\underline{B}$. cervicata are closely related and similar to $\underline{B}$. sandvicensis. Bidens micrantha, B. menziesii, and $\underline{B}$. torta form a third
morphological group, while both $\underline{B}$. hawaiensis and $\underline{B}$. populifolia are relatively distinct from all other taxa. None of these relationships are evident in isozyme data. Although one population or another seems to be separated from the others in a given PCA plot or in the dendrogram, they vary from analysis to analysis, and the genetic distances in the dendrogram and the proportion of variation dealt with by the PCA are much too small for any conclusion except that there is no evidence of well-marked differentiation in isozymes.

Another pattern in the genetic identities is geographical. Most taxa are either endemic to one island or are found on adjacent islands, suggesting that interisland colonizations are fairly rare (Table 16). In conjuction with the fact that subspecies of one species are usually found on different islands, this implies that divergence and speciation events often occur after dispersal to an adjacent island. Comparison of populations using genetic identity values supports this hypothesis. If intrataxon comparisons are removed from the analysis, the genetic identities for populations found on adjacent islands are significantly higher than for comparisons involving populations on the same island or on distant islands. Moreover, even the genetic identities for comparisons of populations from distant islands exceed those for populations on the same island. Thus the most closely related taxa occur on adjacent islands. This suggests that island-hopping may often result in only slight differentiation of populations. If populations diverge sufficiently to be named different taxa, but
table 16. DIStribution of bidens taxa on the hawailan islands.

| TAXON | NI IHAU | KAUAI | OAHU | MOLOKAI | LANAI | MAUI | HAWA I I |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Bidens amplectens |  |  | X |  |  |  |  |
| Bidens asymmetrica |  |  | $x$ |  |  |  |  |
| Bidens campylotheca campylotheca |  |  | X |  | $x$ |  | $x$ |
| Bidens campylotheca pentamera |  |  |  |  |  | $x$ |  |
| Bidens campylotheca waihoiensis |  |  |  | . |  | X |  |
| Bidens ceryicata | $x$ | $x$ | $x$ |  |  |  |  |
| Bidens conjuncta |  |  |  |  |  | $x$ |  |
| Bidens cosmoides |  | $x$ |  |  |  |  |  |
| Bidens forbesii forbesii |  | $x$ |  |  |  |  |  |
| Bidens forbesii kahiliensis |  | $x$ |  |  |  |  |  |
| Bidens havaiensis |  |  |  |  |  |  | $x$ |
| Bidens hillebrandiana hillebrandiana |  |  |  |  |  |  | $x$ |
| Bidens hillebrandiana polycephala |  |  |  | $x$ |  | x |  |
| Bidens macrocarpa |  |  | $x$ |  |  |  |  |
| Bidens mauiensis |  |  |  |  | $x$ | $x$ |  |
| Bidens menziesii menziesii |  |  |  | X |  | $x$ |  |
| Bidens menziesii filiformis |  |  |  |  |  |  | $x$ |
| Bidens micrantha micrantha |  |  |  |  | X | X |  |
| Bidens micrantha ctenophylia | . |  |  |  |  |  | X |
| Bidens micrantha kalealaha |  |  |  |  |  | $x$ |  |
| Bidens molokaiensis |  |  | $x$ | $x$ |  |  |  |
| Bidens populifolia |  |  | $x$ |  |  |  |  |
| Bidens sandvicensis sandvicensis |  | $x$ | $x$ |  |  |  |  |
| Bidens sandvicensis confusa |  | X |  |  |  |  |  |
| Bidens torta. |  |  | $X$ |  |  |  |  |
| Bidens valida |  | $x$ |  |  |  |  |  |
| Bidens wiebkei |  |  |  | $x$ |  |  |  |

not enough to change allele frequencies at isozyme loci appreciably, then intertaxon genetic identities would be expected to be highest for comparisons of populations on different islands.

Examination of polyacetylenes has revealed that Hawaiian Bidens produce a limited array of these compounds compared to continental species. Most of the taxa surveyed for isozymes either have no polyacetylenes in their leaves or produce minute amounts, although the roots of the different taxa do have one common polyacetylene (Marchant, pers. comm.). The polyacetylene data are in agreement with the isozyme data in showing no correlation with taxonomic or morphological divergence of the taxa, but isozymes are much more variable than the polyacetylenes.

Comparisons of Hawaiian Bidens with American taxa yields genetic distance values expected for interspecific comparisons, and principal components analysis separates the two groups without any overlap. The pattern of genetic differentiation of the Hawaiian populations can therefore be considered distinctive to the islands and not characteristic of the genus. Although Bidens amplissima and $B$. tripartita have a high genetic identity, they are also very similar morphologically and are probably very closely related. Aside from this instance, all comparisons of Hawaiian with American taxa or comparisons of American taxa with each other give genetic identity values much lower than for comparisons of Hawaiian populations among themselves.

Patterns of differentiation analogous to that in Hawaian Bidens are found in other groups of unrelated organisms that have undergone recent adaptive radiation. Both the silversword alliance and Lipochaeta on Hawaii exhibit patterns of speciation similar to that found in Hawaiian Bidens. The three genera of the silversword alliance (Argyroxiphium, Dubautia, and Wilkesia) exhibit great morphological and ecological diversity, yet retain the capacity to interbreed (Carr and Kyhos, 1981). Interspecific and even intergeneric hybrids occur commonly in nature. However, there is much cytological diversity as well, including both reciprocal translocations and aneuploidy. The two Hawaiian sections of Lipochaeta have also undergone adaptive radiation in a number of morphological characters (Gardner, 1976; Rabakonandrianina, 1980). Both intrasectional and intersectional hybrids can be obtained, although intersectional hybrids have abnormal meiosis because of the different ploidy levels of the two sections. Intrasectional hybrids are fully fertile. As in Bidens, little natural hybridization occurs between species because of ecological differences between them and the rarity of habitat juxtaposition. Speciation appears to have occurred on separate islands in the tetraploid section but on the same island within the diploid section (Gardner, 1976). Unfortunately, no data on isozymes are available for these genera. Isozyme data are available for Tetramolopium, however, another genus that has undergone adaptive radiation in Hawaii. As in Hawaiian Bidens, all species are similar at the loci studied (Crawford, pers. comm.).

Hawaian Drosophila have been extensively studied morphologically, behaviourally, cytogenetically and electrophoretically. There is a contrast between patterns of speciation in Hawaii and on the mainland in this genus. Continental Drosophila evolution involves speciation in which inversion differences between species prevent successful interbreeding, and different species do not show marked morphological and behavioural differences (Ayala et al., 1974).

In Hawaii, on the other hand, there are great morphological and behavioural differences with very little concomitant cytogenetic differentiation (Carson et al., 1970). Isozyme studies on continental species have revealed genetic differentiation in isozymes with genetic identities conforming to the taxonomic relationships of populations and to the degree of morphological divergence observed (Ayala et al., 1974; Avise, 1976). Hawaiian taxa, however, are in general very similar to each other at isozyme loci although levels of polymorphism within populations are similar to those found in continental populations (Carson and Johnson, 1975; Carson et al., 1975; Johnson et al., 1975; Carson and Kaneshiro, 1976; Sene and Carson, 1977; Craddock and Johnson, 1979). As in Hawaiian Bidens, absence of genetic variability cannot explain the lack of isozyme differentiation among different taxa. Unlike Bidens, the Hawaiian Drosophila species are reproductively isolated from each other because of behavioural mechanisms. This may not represent a more profound genetic difference between species than the reproductive isolation of Bidens species in different habitats although it is
probably a more rigorous mechanism of isolation. The extent of morphological diversity relative to mainland species, the low level of cytogenetic differentiation, and the absence of genetic differentiation in structural genes seem to be common to adaptive radiation on oceanic islands.

The example of island speciation most similar to Bidens is found in Partula (Johnson, 1977; Murray and Clarke, 1980). Partula is a genus of land snails consisting of 9 taxa on the island of Moorea in French Polynesia. The group is highly polymorphic morphologically, yet is homogeneous chromosomally. The taxa are capable of interbreeding, although not in all possible combinations. Sympatric species remain distinct from each other possibly through behavioural differences. No taxon is reproductively isolated from all others, however, so it is theoretically possible for an allele to be passed from any one taxon to any other. The polymorphic species are mainly outbreeding but the snails are capable of self-fertilization. All the Moorean taxa are probably descendants of a single introduction of the genus to the island, and colonization of other nearby islands occurred from Moorea. The isozyme data also show high polymorphism within populations but, in contrast with morphological data, little differentiation among populations has occurred. All the Moorean taxa could be considered one species if a taxonomy of the group were based only on isozyme data. A mean genetic identity of 0.91 was obtained for conspecific populations with a range of 0.89 to 0.95, and the mean for interspecific comparisons was identical:
0.91 with a range of 0.84 to 0.98 . Finally, when comparisons of Moorean and Tahitian taxa are made, it is evident that the most closely related species occur on different islands. It seems likely that colonization of other islands may lead to speciation, but with relatively little initial differentiation. Species showing more divergence may have been isolated from each other for a longer period. Although speciation could occur as a consequence of colonization of a different part of the same island as well, the degree of genetic isolation conferred by interisland colonizations may make this a more common method of speciation.

It is evident that island genera in which adaptive radiation has occurred share many similarities in patterns of differentiation and speciation regardless of whether they are plants or animals. Great morphological diversity, a mechanism preventing interbreeding between populations (whether behavioural ar ecological), little chromosomal evolution, and little divergence at isozyme loci distinguish these situations from patterns of continental evolution. The similarities of Polynesian land snails, fruit flies, and beggar's ticks in patterns of evolutionary diversification are much greater than their similarities with their continental relatives. The underlying mechanisms that have caused the patterns may be different in these groups, however. In Hawaiian Drosophila, the rapid rates of speciation have been attributed to founder effects which radically increase inbreeding and cause gametic disequilibrium in only a small subset of the genetic variability
found in the ancestral population (Mayr, 1954, 1955). This may alter selective values of genes and favour the alleles which are compatible in homozygous form with certain alleles at other loci. Carson (1970, 1975) limited this argument to only the "closed" portion of the genome (not including genes coding for isozymes) after the electrophoretic similarity of most Hawaiian species was discovered. Recent theories incorporate sexual selection arguments (Spieth, 1974) with founder effect models to explain the extensive morphological and behavioural diversity of Drosophila on Hawaii (Templeton, 1980a, 1980b). Explanations relying on effects of inbreeding and sexual selection are not convincing for Bidens species, in which inbreeding is common and sexual selection, if present at all, is confined to gynodioecy in some taxa. They may not even apply to Partula completely because inbreeding is not uncommon in snails, either. The morphological and ecological differentiation in Bidens consists of characters of more direct adaptive value to the physical environment than the differentiation of Drosophila which is related to sexual selection. Although some unified explanation may yet account for all instances of island speciation, it seems more likely that different evolutionary mechanisms have produced a similar pattern of speciation.

Hawaiian Bidens are highly differentiated morphologically and ecologically, but they are chromosomally homogeneous and retain the capacity to interbreed. Although the differences between taxa are genetically controlled, the genetic divergence does not extend to structural gene loci. Populations are more
variable than most plant populations, but little genetic differentiation has occurred in this portion of the genome. Populations that are very similar morphologically and are classified in the same taxon are also very similar genetically, but the correlation does not extend to intertaxon comparisons. Populations of different species are as similar as populations belonging to different subspecies, and no groups of species are evident in the isozyme data although morphological groups exist. Genetic differentiation at isozyme loci has not occurred at the same rate as the acquisition of adaptive morphological and ecological characters in Hawaiian Bidens. Adaptive radiation can thus occur without extensive changes throughout the genome, and may in fact involve relatively few genes.

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Appendix A. COMPLETE SCIENTIFIC NAMES OF BIDENS TAXA STUDIED.

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Hawaiian Taxa
B. asymmetrica (Levl.) Sherff
B. cervicata Sherff
B. forbesii Sherff ssp. forbesii
B. hawaiensis (Gray) Sherff
B. mauiensis (Gray) Sherff
B. menziesif (Gray) Sherff ssp. filiformis (Sherff) Ganders and Nagata
B. micrantha Gaud. ssp. micrantha
B. micrantha Gaud. ssp. Ctenophylla (Sherff) Nagata and Ganders
B. micrantha Gaud. ssp. kalealaha Nagata and Ganders
B. molokaiensis (Hillebr.) Sherff
B. populifolia Sherff
B. sandvicensis Less. ssp. sandvicensis
B. Sandvicensis Less. ssp. Confusa Nagata and Ganders
B. torta Sherff
B. Wiebkei Sherff
American Taxa
B. amplissima Greene
B. Cynapifolia H.B.K.
B. frondosa L.
B. tripartita L.
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APPENDIX B. ALLELE FREQUENCIES IN BIDENS POPULATIONS STUDIED.

| ALLELE | $\begin{aligned} & \text { ASYM } \\ & \text { B4 } \end{aligned}$ | $\begin{aligned} & \text { CERV } \\ & \text { B87 } \end{aligned}$ | $\begin{aligned} & \text { FORB } \\ & \text { B14 } \end{aligned}$ |  | $\begin{aligned} & \text { HAWA } \\ & \text { B23. } \end{aligned}$ | $\begin{aligned} & \text { HAWA } \\ & \text { B50 } \end{aligned}$ | $\begin{aligned} & \text { MENZ } \\ & \text { B1O9 } \end{aligned}$ | F | $\begin{aligned} & \text { MENZ } \\ & \text { B } 130 \end{aligned}$ | F | MENZ <br> B218 | F | $\begin{aligned} & \text { MENZ } \\ & \text { B2 } 19 \end{aligned}$ | F | $\begin{aligned} & \text { MICR } \\ & \text { B78 } \end{aligned}$ |  | $\begin{aligned} & \text { MICR } \\ & \text { B149 } \end{aligned}$ | C | $\begin{aligned} & \text { MICR K } \\ & \text { B197 } \end{aligned}$ | $\begin{aligned} & \text { MOLO } \\ & \text { B72 } \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGI-1a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 |
| PGI-2a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 |
| PGI-3a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 |
| PGI-3b | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-4a | 0.17 | 0.00 | 0.00 |  | 0.00 | 0.09 | 0.00 |  | 0.01 |  | 0.05 |  | 0.00 |  | 0.00 |  | 0.08 |  | 0.00 | 0.00 |
| PGI-4b | 0.33 | 0.03 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.01 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-4C | 0.02 | 0.82 | 0.02 |  | 0.12 | 0.07 | 0. 19 |  | 0.34 |  | 0.05 |  | 0.17 |  | 0.24 |  | 0.03 |  | 0.20 | 0.00 |
| PGI-4d | 0.48 | 0. 15 | 0.94 |  | 0.49 | 0.31 | 0.80 |  | 0.64 |  | 0.86 |  | 0.83 |  | 0.76 |  | 0.90 |  | 0.80 | 1.00 |
| PGI-4e | 0.00 | 0.00 | 0.04 |  | 0.02 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-4f | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.01 | 0.01 |  | 0.00 |  | 0.04 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-4g | 0.00 | 0.00 | 0.00 |  | 0.36 | 0.51 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-4k | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-41 | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-4n | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-5a | 0.00 | 0.01 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-5b | 0.00 | 0.01 | 0.00 |  | 0.00 | 0.01 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.01 |  | 0.00 | 0.00 |
| PGI-5c | 0.81 | 0.60 | 1.00 |  | 0.74 | 0.45 | 0.75 |  | 0.54 |  | 0.78 |  | 0.61 |  | 0.76 |  | 0.85 |  | 0.95 | 1.00 |
| PGI-5d | 0.00 | 0.26 | 0.00 |  | 0.25 | 0.52 | 0.24 |  | 0.41 |  | 0.20 |  | 0.39 |  | 0. 19 |  | 0.11 |  | 0.00 | 0.00 |
| PGI-5e | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.01 |  | 0.00 | 0.00 |
| PGI-5i | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-5j | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-5k | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGI-5n | 0.19 | 0.12 | 0.00 |  | 0.01 | 0.01 | 0.01 |  | 0.04 |  | 0.02 |  | 0.00 |  | 0.05 |  | 0.01 |  | 0.05 | 0.00 |
| PGM-1a | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.06 |  | 0.02 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-1b | 0.77 | 0.96 | 0.78 |  | 1.00 | 0.96 | 0.94 |  | 0.47 |  | 0.50 |  | 0.53 |  | 0.90 |  | 0.21 |  | 0.95 | 0.98 |
| PGM-1C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.43 |  | 0.26 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-1d | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.04 |  | 0.04 |  | 0.18 |  | 0.39 |  | 0.10 |  | 0.79 |  | 0.05 | 0.02 |
| PGM-19 | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 0.00 |  | 0.00 | 0.00 |
| PGM-1 C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-1n | 0.23 | 0.04 | 0.22 |  | 0.00 | 0.04 | 0.02 |  | 0.00 |  | 0.03 |  | 0.08 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-2a | 0.00 | 0.00 | 0.04 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.01 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-2b | 1.00 | 1.00 | 0.96 |  | -0.89 | 0.68 | 0.96 |  | 1.00 |  | 0.98 |  | 0.98 |  | 0.85 |  | 0.98 |  | 1.00 | 1.00 |
| PGM-2C | 0.00 | 0.00 | 0.00 |  | 0.10 | 0.31 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-2d | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.02 |  | 0.00 | 0.00 |
| PGM-2f | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-2g | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 0.00 |
| PGM-2h | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 0.02 |  | 0. 0.15 |  | 0.00 |  | 0.00 | 0.00 |
| PGM-2n | 0.00 0.00 | 0.00 0.00 | 0.00 0.00 |  | 0.00 0.00 | 0.01 0.00 | 0.04 0.28 |  | 0.00 0.18 |  | 0.01 0.09 |  | 0.02 0.00 |  | 0.15 0.18 |  | 0.49 |  | 0.22 | 0.00 |
| PGM-3a PGM-3b | 0.00 1.00 | 0.00 0.99 | 0.00 0.96 |  | 0.00 0.99 | 0.01 1.00 | 0.28 0.72 |  | 0.80 |  | 0.91 |  | 0.93 |  | 0.82 |  | 0.51 |  | 0.78 | . 0.97 |
| PGM-3n | 0.00 | 0.01 | 0.04 |  | 0.01 | 0.00 | 0.00 |  | 0.01 |  | 0.00 |  | 0.07 |  | 0.00 |  | 0.00 |  | 0.00 | 0.03 |
| PGM-4a | 0.58 | 0.88 | 0.74 |  | 0.76 | 0.36 | 0.92 |  | 0.68 |  | 0.63 |  | 0.81 |  | 0.90 |  | 0.61 |  | 0.50 | 0.93 |


| ALLELE | $\begin{aligned} & \text { ASYM } \\ & \text { B4 } \end{aligned}$ | $\begin{aligned} & \text { CERV } \\ & \text { B87 } \end{aligned}$ | FORB $B \cdot 14$ |  | $\begin{aligned} & \text { HAWA } \\ & \text { B231 } \end{aligned}$ | HAWA <br> B50 | $\begin{aligned} & \text { MENZ } \\ & \text { B109 } \end{aligned}$ | F | MENZ <br> B130 | F | $\begin{aligned} & \text { MENZ } \\ & \text { B2 } 18 \end{aligned}$ | F | MENZ <br> B2 19 | F | $\begin{aligned} & \text { MICR } \\ & \text { B78 } \end{aligned}$ |  | $\begin{aligned} & \text { MICR } \\ & \text { B149 } \end{aligned}$ | C | $\begin{aligned} & \text { MICR } \\ & \text { B } 197 \end{aligned}$ | K | $\begin{aligned} & \text { MOLO } \\ & 872 \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGM-4b | 0.00 | 0.00 | 0.13 |  | 0.07 | 0.13 | 0.00 |  | 0.01 |  | 0.07 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| PGM-4C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.01 |  | 0.00 |  | 0.00 |
| PGM-4n | 0.42 | 0.12 | 0.13 |  | 0.17 | 0.51 | 0.08 |  | 0.30 |  | 0.30 |  | 0.19 |  | 0.10 |  | 0.39 |  | 0.50 |  | 0.07 |
| LAP-1a | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| LAP-1b | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| LAP-1C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.06 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.07 |  | 0.00 |
| LAP-1d | 1.00 | 1.00 | 1.00 |  | 0.95 | 0.96 | 0.90 |  | 0.95 |  | 1.00 |  | 1.00 |  | 0.98 |  | 0.92 |  | 0.60 |  | 1.00 |
| $L \triangle P-1 e$ | 0.00 | 0.00 | 0.00 |  | 0.05 | 0.04 | 0.03 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.33 |  | 0.00 |
| LAP-1n | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.01 |  | 0.05 |  | 0.00 |  | 0.00 |  | 0.02 |  | 0.08 |  | 0.00 |  | 0.00 |
| LAP-2a | 0.00 | 0.00 | 0.00 |  | 0.16 | 0.15 | 0.04 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.02 |  | 0.00 |  | 0.29 |  | 0.00 |
| LAP-2b | 0.11 | 1.00 | 1.00 |  | 0.56 | 0.60 | 0.74 |  | 0.96 |  | 0.92 |  | 1.00 |  | 0.92 |  | 0.61 |  | 0.71 |  | 0.56 |
| LAP-2C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| LAP-2d | 0.85 | 0.00 | 0.00 |  | 0.24 | 0.24 | 0.00 |  | 0.00 |  | 0.06 |  | 0.00 |  | 0.00 |  | 0.37 |  | 0.00 |  | 0.44 |
| LAP-2e | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.03 |  | 0.00 |  | 0.02 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| LAP-2n | 0.04 | 0.00 | 0.00 |  | 0.04 | 0.01 | 0.19 |  | 0.04 |  | 0.00 |  | 0.00 |  | 0.05 |  | 0.02 |  | 0.00 |  | 0.00 |
| DIA-1a | 0.11 | 0.76 | 0.07 |  | 0.00 | 0.12 | 0.92 |  | 0.95 |  | 1.00 |  | 0.95 |  | 0.16 |  | 0.89 |  | 0.41 |  | 0.92 |
| DIA-10 | 0.87 | 0.24 | 0.37 |  | 1.00 | 0. 88 | 0.08 |  | 0.05 |  | 0.00 |  | 0.05 |  | 0.58 |  | 0.11 |  | 0.02 |  | 0.08 |
| DIA-1C | 0.02 | 0.00 | 0.07 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.26 |  | 0.00 |  | 0.39 |  | 0.00 |
| DIA-1d | 0.00 | 0.00 | 0.50 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.18 |  | 0.00 |
| DIA-1e | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 1.00 |
| DIA-2a | 1.00 | 0.97 | 0.50 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 0.99 |  | 1.00 |  | 1.00 |
| DIA-2b | 0.00 | 0.03 | 0.50 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.01 |  | 0.00 |  | 0.00 |
| DIA-2C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| MDH-1a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |
| MDH-2a | 0.00 | 0.00 | 0.12 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| MDH-2 | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.32 |  | 0.12 |  | 0.02 |  | 0.00 |  | 0.00 |  | 0.02 |  | 0.00 |  | 0.00 |
| MDH-2C | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |
| MDH-2d | 1.00 | 1.00 | 0.88 |  | 1.00 | 1.00 | 0.68 |  | 0.88 |  | 0.98 |  | 1.00 |  | 1.00 |  | 0.98 |  | 1.00 |  | 1.00 0.97 |
| MDH-3a | 0.34 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.21 |  | 0.44 |  | 0.63 |  | 0.41 |  | 0.37 |  | 0.69 |  | 0.00 |  | 0.97 |
| MDH-3b | 0.66 | 1.00 | 1.00 |  | 1.00 | 1.00 | 0.79 |  | 0.56 |  | 0.37 |  | 0.59 |  | 0.63 |  | 0.31 |  | 1.00 |  | 0.03 |
| MDH-4a | 1.00 | 1. 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 0.86 |  | 1.00 0.97 |
| MDH-5a | 1.00 | 0.96 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 0.96 |  | 0.98 |  | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 0.86 0.00 |  | 0.97 0.00 |
| MDH-5b | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 0.00 |  | 0.00 0.14 |  | 0.00 0.00 |
| MDH-5c | 0.00 | 0.04 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.04 |  | 0.02 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.14 |  | 0.00 |
| MDH-5d | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.03 |
| MDH-5e | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 -1.00 |
| MDH-6a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 0.93 |  | -1.00 |
| MDH-6b | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.07 |  | 0.00 |
| $\times \mathrm{OH}-1 \mathrm{a}$ | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 1.00 |  | 1.00 1.00 |  | 1.00 1.00 |
| ME - 1a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 .00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 |
| ME - 1 b | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 1.00 |  | 1.00 1.00 |  | 1.00 1.00 |
| HA - 1 a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 0.00 |
| HA - 1 b | 0.00 | 0.00 | 0.00 |  | 0.00 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 |  | 1.00 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |
| GLU-1a | 1.00 | 1.00 | 1.00 |  | 1.00 | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 |


| ALLELE | $\begin{aligned} & \text { POPU } \\ & \text { B42 } \end{aligned}$ | $\begin{aligned} & \text { SAND } \\ & \text { B44 } \end{aligned}$ | S | $\begin{aligned} & \text { SAND } \\ & \text { B2OO } \end{aligned}$ | S | $\begin{aligned} & \text { SAND } \\ & \text { B34 } \end{aligned}$ |  | $\begin{aligned} & \text { TORT } \\ & \text { B37 } \end{aligned}$ | $\begin{aligned} & \text { TORT } \\ & \text { B15 } \end{aligned}$ | $\begin{aligned} & \text { TORT } \\ & \text { B2 } 13 \end{aligned}$ | $\begin{aligned} & \text { TORT } \\ & 8257 \end{aligned}$ | WIEB B260 | AMPL | TRIP | FRON | CYNA |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGI-1a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI-2a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI-3a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-3b | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI-4a | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4b | 0.00 | 0.00 |  | 0.03 |  | 0.00 |  | 0.04 | 0.00 | 0.02 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4C | 0.22 | 0.00 |  | 0.06 |  | 0.07 |  | 0. 12 | 0.11 | 0.27 | 0.31 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4d | 0.78 | 1.00 |  | 0.90 |  | 0.93 |  | 0.80 | 0.88 | 0.69 | 0.65 | 0.92 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4e | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4f | 0.00 | 0.00 |  | 0.01 |  | 0.00 |  | 0.05 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4g | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4k | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| PGI-41 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGI-4n | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.01 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5a | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5b | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.02 | 0.13 | 0.03 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5c | 1.00 | 1.00 |  | 0.99 |  | 0.93 |  | 0.91 | 0.87 | 0.85 | 0.88 | 0.98 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5d | 0.00 | 0.00 |  | 0.00 |  | 0.05 |  | 0.06 | 0.00 | 0.11 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5e. | 0.00 | 0.00 |  | 0.01 |  | 0.02 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | . 0.00 |
| PGI-5i | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 0.00 |
| PGI-5j | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGI-5K | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| PGI-5n | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.01 | 0.00 | 0.02 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1a | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1b | 1.00 | 0.98 |  | 0.86 |  | 0.88 |  | 1.00 | 1.00 | 1.00 | 0.98 | 0.98 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1c | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1d | 0.00 | 0.00 |  | 0.01 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-19 | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 0.00 | 0.00 1.00 |
| PGM-in | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGM-in | 0.00 | 0.02 |  | 0.13 |  | 0.12 |  | 0.00 | 0.00 | 0.00 | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2a | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2b | 1.00 | 1.00 |  | 0.86 |  | 0.98 |  | 1.00 | 1.00 | 0.90 | 0.93 | 0.92 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2c | 0.00 | 0.00 |  | 0.14 |  | 0.00 |  | 0.00 | 0.00 | 0.06 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 0.00 |
| PGM-2d | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2f | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | -0.00 | 0.00 |
| PGM-2g | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 0.00 | 1.00 0.00 | 0.00 1.00 |
| PGM-2h | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 0.04 | 0.00 0.07 | 0.00 0.00 | 0.00 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2n PGM-3a | 0.00 0.00 | 0.00 0.41 |  | 0.00 0.01 |  | 0.02 0.00 |  | 0.00 0.09 | 0.00 0.01 | 0.04 0.04 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-3b | 0.98 | 0.59 |  | 0.96 |  | 0.90 |  | 0.88 | 0.99 | 0.93 | 0.90 | 0.93 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGM-3n | 0.02 | 0.00 |  | 0.03 |  | 0.10 |  | 0.04 | 0.00 | 0.04 | 0.08 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4a | 0.93 | 1.00 |  | 0.97 |  | 0.90 |  | 0.86 | 0.90 | 0.80 | 0.91 | 0.72 | 0.00 | 0.00 | 0.00 | 0.00 |


| ALLELE | $\begin{aligned} & \text { POPU } \\ & \text { B42 } \end{aligned}$ | $\begin{aligned} & \text { SAND } \\ & \text { B44 } \end{aligned}$ |  | $\begin{aligned} & \text { SAND } \\ & \text { B200 } \end{aligned}$ | S | $\begin{aligned} & \text { SAND } \\ & \text { B34 } \end{aligned}$ |  | $\begin{aligned} & \text { TORT } \\ & \text { B37 } \end{aligned}$ | $\begin{aligned} & \text { TORT } \\ & \text { B } 15 \end{aligned}$ | $\begin{aligned} & \text { TORT } \\ & \text { B2 } 13 \end{aligned}$ | $\begin{aligned} & \text { TORT } \\ & \text { B257 } \end{aligned}$ | WIEB <br> B260 | $\triangle M P L$ | TRIP | FRON | CYNA |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGM-4b | 0.00 | 0.00 |  | 0.01 |  | 0.00 |  | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4C | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4n | 0.07 | 0.00 |  | 0.02 |  | 0.10 |  | 0.14 | 0.10 | 0.18 | 0.09 | 0.02 | 1.00 | 1.00 | 1.00 | 1.00 |
| LAP-1a | 0.00 | 0.00 |  | 0.02 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-tb | 0.00 | 0.00 |  | 0.08 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1C | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.10 | 0.31 | 0.00 | 0.19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1d | 1.00 | 1.00 |  | 0.90 |  | 0.94 |  | 0.83 | 0.69 | 0.94 | 0.74 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1e | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1n | 0.00 | 0.00 |  | 0.00 |  | 0.06 |  | 0.06 | 0.00 | 0.06 | 0.02 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| LAP-2a | 0.00 | 0.00 |  | 0.17 |  | 0.00 |  | 0.03 | 0.00 | 0.06 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2b | 0.81 | 0.93 |  | 0.82 |  | 0.81 |  | 0.86 | 1.00 | 0.90 | 0.75 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| LAP-2C | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2d | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.02 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2e | 0. 19 | 0.00 |  | 0.00 |  | 0.00 |  | 0.04 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2n | 0.00 | 0.07 |  | 0.02 |  | 0. 19 |  | 0.06 | 0.00 | 0.04 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1a | 0.40 | 0.50 |  | 0.90 |  | 0.68 |  | 0.96 | 1.00 | 0.31 | 0.34 | 0.88 | 0.00 | 0.00 | 1.00 | 0.00 |
| DIA-1b | 0.60 | 0.50 |  | 0.10 |  | 0.32 |  | 0.04 | 0.00 | 0.60 | 0.53 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1c | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.07 | 0.09 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1d | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1e | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 1.00 |
| DIA-2a | 0.83 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 0.97 | 0.98 | 1.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| DIA-2b | 0.17 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.03 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-2C | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 1.00 |
| MDH-1a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-2a | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-2b | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $\mathrm{MDH}-2 \mathrm{C}$ | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-2d | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 0.91 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-3a | 0.77 | 0.93 |  | 0.55 |  | 0.79 |  | 0.00 | 0.07 | 0.00 | 0.00 | 0.37 | 1.00 | 1.00 | 1.00 | 1.00 0.00 |
| MDH-3b | 0.23 | 0.07 |  | 0.45 |  | 0.21 |  | 1.00 | 0.93 | 1.00 | 1.00 | 0.63 | 0.00 | 0.00 | 1.00 1.00 | 0.00 |
| MDH-4a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MOH-5a | 1.00 | 0.98 |  | 0.95 |  | 0.48 |  | 0.95 | 0.61 | 0.96 | 0.91 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-5b | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-5c | 0.00 | 0.02 |  | 0.04 |  | 0.38 |  | 0.05 | 0.39 | 0.04 | 0.09 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-5d | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-5e | 0.00 | 0.00 |  | 0.01 |  | 0.14 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-6a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-6b | 0.00 | 0.00 |  | 0.00 |  | 0.00 |  | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $\times \mathrm{DH}-1 \mathrm{a}$ | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 1.00 | 1.00 1.00 | 1.00 1.00 | 1.00 0.00 |
| ME - 1 a | 1. 000 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 1.00 |
| ME - 1 b | 1.00 1.00 | 1.00 1.00 |  | 0.00 1.00 |  | 1.00 1.00 |  | 1.00 1.00 | 0.00 1.00 | 0.00 1.00 | 1.00 0.00 1.00 | 1.00 1.00 | 1.00 1.00 | 1.00 1.00 | 1.00 1.00 | 0.00 |
| HA - 1 a HA - 10 | 1.00 0.00 | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 |  | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 1.00 0.00 | 0.00 1.00 |
| GLU-1a | 1.00 | 1.00 |  | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

APPENDIX C. ALLELE FREQUENCIES IN BIDENS TAXA STUDIED.



| Allele | POPU | SAND | S | SAND | C | TORT | WIEB | AMPL | TRIP | FRON | CYNA |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| PGI-1a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI-2a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI-3a | 1.00. | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-3b | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGI-4a | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4b | 0.00 | 0.01 |  | 0.00 |  | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4c | 0.21 | 0.05 |  | 0.07 |  | 0.24 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4d | 0.79 | 0.94 |  | 0.91 |  | 0.72 | 0.90 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4e | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4f | 0.00 | 0.01 |  | 0.02 |  | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4g | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-4k | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| PGI-41 | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGI-4n | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5a | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5b | 0.00 | 0.00 |  | 0.00 |  | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5c | 1.00 | 0.99 |  | 0.93 |  | 0.87 | 0.99 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5d | 0.00 | 0.00 |  | 0.04 |  | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5e | 0.00 | 0.01 |  | 0.02 |  | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGI-5i. | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 0.00 |
| PGI-5j | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGI-5k | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| PGI-5n | 0.00 | 0.00 |  | 0.00 |  | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1a | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1b | 1.00 | 0.90 |  | 0.89 |  | 0.97 | 0.97 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1c | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-1d | 0.00 | 0.01 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-19 | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 1.00 | 1 :00 | 0.00 |
| PGM-1h | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGM-1n | 0.00 | 0.10 |  | 0.11 |  | 0.02 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2a | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2b | 1.00 | 0.91 |  | 0.98 |  | 0.94 | 0.90 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2c | 0.00 | 0.08 |  | 0.00 |  | 0.03 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2d | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-2f | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 0.00 | 0.00 | 0.00 |
| PGM-2g | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 |
| PGM-2h | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| PGM-2n | 0.00 | 0.01 |  | 0.02 |  | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-3a | 0.00 | 0.10 |  | 0.00 |  | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-3b | 0.98 | 0.86 |  | 0.91 |  | 0.93 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 |
| PGM-3n | 0.02 | 0.04 |  | 0.09 |  | 0.03 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4a | 0.93 | 0.96 |  | 0.91 |  | 0.81 | 0.64 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4b | 0.00 | 0.01 |  | 0.00 |  | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4C | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.34 | 0.00 | 0.00 | 0.00 | 0.00 |
| PGM-4n | 0.07 | 0.03 |  | 0.09 |  | 0.18 | 0.01 | 1.00 | 1.00 | 1.00 | 1.00 |


| ALLELE | POPU | SAND | S | SAND | C | TORT | WIEB | AMPL | TRIP | FRON | CYNA |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| LAP-1a | 0.00 | 0.01 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $L A P-1 b$ | 0.00 | 0.07 |  | 0.00 |  | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1C | 0.00 | 0.01 |  | 0.00 |  | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1d. | 1.00 | 0.90 |  | 0.95 |  | 0.83 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-ie | 0.00 | 0.00 |  | 0.00 |  | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-1n | 0.00 | 0.00 |  | 0.05 |  | 0.05 | 0.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| LAP-2a | 0.00 | 0.10 |  | 0.02 |  | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2b | 0.82 | 0.87 |  | 0.80 |  | 0.81 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| LAP-2C | 0.00 | 0.00 |  | 0.00 |  | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2d | 0.00 | 0.00 |  | 0.00 |  | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $L A P-2 e$ | 0.18 | 0.00 |  | 0.00 |  | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| LAP-2n | 0.00 | 0.04 |  | 0.18 |  | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1a | 0.38 | 0.71 |  | 0.70 |  | 0.46 | 0.90 | 0.00 | 0.00 | 1.00 | 0.00 |
| DIA - 1b | 0.62 | 0.29 |  | 0.30 |  | 0.46 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1c | 0.00 | 0.00 |  | 0.00 |  | 0.06 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1d | 0.00 | 0.00 |  | 0.00 |  | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-1e | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | 1.00 |
| DIA-2a | 0.84 | 0.98 |  | 1.00 |  | 0.98 | 1.00 | 0.00 | 0.00 | 1.00 | 0.00 |
| DIA-2b | 0.16 | 0.02 |  | 0.00 |  | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| DIA-2c | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 1.00 | 1.00 | 0.00 | -1.00 |
| MDH-1a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-2a | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-2b | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-2c | 0.00 | 0.00 |  | 0.00 |  | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-2d | 1.00 | 1.00 |  | 1.00 |  | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-3a | 0.78 | 0.60 |  | 0.72 |  | 0.01 | 0.32 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-3b | 0.22 | 0.40 |  | 0.28 |  | 0.99 | 0.68 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-4a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-5a | 1.00 | 0.94 |  | 0.50 |  | 0.88 | 0.90 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-5b | 0.00 | 0.00 |  | 0.80 |  | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-5c | 0.00 | 0.04 |  | 0.36 |  | 0.12 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-5d | 0.00 | 0.00 |  | 0. 14 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-5e | 0.00 | 0.02 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| MDH-6a | 1.00 | 1.00 |  | 1.00 |  | 0.99 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| MDH-6c | 0.00 | 0.00 |  | 0.00 |  | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| $\times \mathrm{DH}-1 \mathrm{a}$ | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| ME - 1 a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| ME -1b | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| HA - 1 a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.00 |
| HA -1b | 0.00 | 0.00 |  | 0.00 |  | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |
| GLU-1a | 1.00 | 1.00 |  | 1.00 |  | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |


[^0]:    ${ }^{1}$ S refers to seeds; the number in brackets is the num

[^1]:    ${ }^{1}$ All gel slices were incubated at $40^{\circ} \mathrm{C}$ except for GLU and HA stains (room
    temperature) and the presoak step for LAP (one hour in refrigerator)
    ${ }^{2}$ Most of the recipes used are modifications of these references.

[^2]:    ${ }^{1}$ Heterozygous $a b$ and homozygous bb progeny were difficult to distinguish. so they are

[^3]:    1 Values are given for loci at which at which the most common allele has a frequency of $\leq 0.99$ or $\leq 0.95$ (in brackets).
    ${ }^{2}$ Values ate given for adeles present at a frequency of $\geq 0.01$ or $\geq 0.05$ (in brackets).

[^4]:    'Genetic identity values are in the upper region of the table, and genetic distances in the lower region.

