# FINAL REPORT

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ENDANGERED AND THREATENED SPECIES CONSERVATION

*Job No. 14*

## Propagation, Genetic Constituion, and Reintroduction of the Hinckley Oak (*Quercus hinckleyi*)

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## ABSTRACT

Initial interpretations of electrophoretic and flavonoid data reveal that interpopulational variability for the Hinckley oak (*Quercus hinckleyi*) is greater than intrapopulational variability. Population sampling indicates a relatively low number of hybridizations of *Q. hinckleyi* X *Quercus pungens* var. *pungens*. Additionally, pollen-stigma analysis does not reveal extreme pollen contamination from other oak species. Seven apparent *Q. hinckleyi* seedlings were observed (for the first time) in the field at the Shafter population. Greenhouse experiments reveal a germination rate of 88%. Micropropagation experiments were unsuccessful and terminated early in the project.

# FINAL REPORT

**STATE:** TEXAS **PROJECT NO.:** E-1-4+5

**PROJECT TITLE:** Endangered and Threatened Species Conservation.

**PERIOD COVERED:** September 1, 1991 through August 31, 1992

**JOB NUMBER:** 41

**JOB TITLE:** Propagation, Genetic Constitution, and Re-introduction of the Hinckley oak (*Quercus hinckleyi*)

**JOB OBJECTIVE:** Investigations in micropropagation and genetic composition of the Hinckley oak are planned to lead to the establishment of other populations within the natural habitat on state property.

**SEGMENT OBJECTIVE:** Continue work on micropropagation and greenhouse propagation. Continue research on the population genetic structure of the Hinckley oak using isozyme, flavonoid, and foliar trichome studies. Search for new populations in Presidio and Brewster counties.

## ACCOMPLISHMENTS

See Attachment

## SIGNIFICANT DEVIATIONS

Dr. Richard Hilsenbeck, the original principal investigator, abandoned this project. As a result, Jackie M. Poole with Texas Parks and Wildlife Department and Sharon Weyerts, a graduate student at Sul Ross State University, established objectives for the year which focused on preliminary ecological surveys of the Hinckley oak populations and continued investigations of putative hybridization with *Quercus pungens* var. *pungens*, a closely related species (see pg. 22, "Remarks" of the attached report).

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# Genetic, Biochemical, and Reproductive Studies of the Hinckley Oak (*Quercus hinckleyi*) in the Big Bend Country of Texas

Final Research Report Submitted to the
Texas Parks and Wildlife Department, Natural Heritage Program
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by

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## INTRODUCTION

The original contract for Quercus hinckleyi research completed during the funding period (Interagency Cooperation Contract, 1990) encompassed the following objectives: 1) To determine the overall pattern of the population genetic structure of Q. hinckleyi populations using starch gel electrophoresis, 2) to determine the extent of hybridization with other oaks in the area using flavonoid chemistry and leaf material from in situ plants as well as cultivated plants (grown from acorns), 3) To examine pollen fertility and pollen loads to assess the amount of gene flow and to determine whether reduced fertility and/or sterility exists, and 4) To develop micropropagation techniques to produce Hinckley oaks for population augmentation or introduction and to avoid genetic dilution. Each year a search for new Hinckley Oak populations in Presidio and Brewster Counties was included. Although not in writing, verbal objectives communicated between Ms. Jackie Poole and myself were incorporated into the research after Dr. Richard Hilsenbeck, the principal investigator, abandoned the project. These verbal objectives focused on preliminary ecological surveys of Hinckley Oak populations and continued investigation of putative hybridization with Q. pungens var. pungens, a closely related species.

## POPULATIONS SAMPLED

Quercus hinckleyi populations in the Solitario were visited eight times during the research period while Q. hinckleyi

populations in Shafter were visited four times. Although Kennedy and Poole (1992) report 10 known populations of Q. hinckleyi, a total of 14 Q. hinckleyi populations in the Solitario have been located and reported to the Texas Parks and Wildlife Department (see Weyerts-1991 Performance Report) and are marked on a topographic map (Fig. 1). In most of the populations, there are from 100-200 healthy Hinckley Oak shrubs; however, cloning makes it difficult to recognize individual plants. Each of the Solitario populations visited occurs at approximately 4200-4400 feet in elevation. Future surveys at this elevation in the Solitario will probably reveal the presence of more Q. hinckleyi populations, particularly trending southeast from the last marked sites in the southwest sector. Two Quercus hinckleyi populations have been located in Shafter, Texas and are not placed on a topographic map in accordance with landowner wishes.

In characterizing the populations sampled, Q. hinckleyi exists in the Solitario on nearly solid limestone with some thin top soil in places, while in the Shafter population the oaks are found in rocky "friable" limestone. Plant species found to be in association with Q. hinckleyi in the Solitario and in Shafter, as reported by Weyerts (1992 Performance Report), are listed in Table 1.

## ELECTROPHORETIC INVESTIGATIONS

Electrophoresis was eventually eliminated from these investigations because of the difficulty in fine-tuning the procedure. Because electrophoresis was so difficult to perfect,

I have included a detailed description of the electrophoretic procedures that I used, should the Texas Parks & Wildlife Department need them for future reference.

Enzyme extraction from the collected leaf material followed the protocol of Soltis et al. (1983) with some slight modifications. Four hundred mg (more or less) of leaf material were placed in a porcelain mortar and pestle and ground under liquid nitrogen until a fine powder was obtained. One to two ml of a phosphate grinding buffer-PVP solution was added to the powder to produce a thick slurry. An additional 1/2-1 ml of phosphate buffer (pH 7.5) was occasionally added so that the liquid could be easily extracted and placed in nunc cryo-tubes. The fluid was then stored in liquid nitrogen.

Two Histidine-Citrate buffer systems and one Lithium-Borate buffer system of Soltis et al. (1983) were initially used for starch gel preparation (Table 2). The day before a run, two gels were poured according to the protocol of Kephart (1990) with some slight modifications. Approximately 1 hr before an electrophoretic run was complete, most of the ingredients for each enzyme stain were mixed so that the enzyme cofactors were the only components necessary to add once the gels were sliced (Kephart, 1990). Each assay solution included: the substrate upon which the enzyme of interest acted; cofactors (NAD and NADP), tetrazolium salts, and coupling enzymes; and a stain that coupled with the product of the reaction (Kephart, 1990; Gottlieb, 1971). Staining schedules varied according to the

buffer system used (Table 3). Twelve enzyme systems known to occur in plants were initially surveyed: Peroxidase (PER), Phosphoglucoisomerase (PGI), Aspartate Aminotransferase (GOT), Leucine Aminopeptidase (LAP), Triosephosphate Isomerase (TPI), 6-Phosphogluconate Dehydrogenase (6-PGD), Shikimate Dehydrogenase (SKDH), Malate Dehydrogenase (MDH), Alcohol Dehydrogenase (ADH), and Phosphoglucomutase (PGM). Upon staining completion, peroxidase gels were "fixed" with a 50% glycerol solution. All other gel slabs were "fixed" in 50% ethanol. If readable gels were obtained, they were scored with regard to $R_f$ values, which were obtained by dividing the distance traveled for each enzyme band by the total distance to the anodal front. Attempts were made to determine levels of genetic variability with respect to the calculated $R_f$ values.

Although Hilsenbeck (1990 Performance Report) reported that Q. hinckleyi is highly homozygous (intrapopulationally) with regard to GOT, MDH, and PER, these data are not valid because the results are not repeatable. Electrophoretic gels for PER, PGM, IDH, 6-PGD, and SKDH, have been photographed and submitted to the Texas Parks and Wildlife Department (see Weyerts, 1991 Performance Report). A cursory examination of these gels indicates that interpopulational variability is greater than intrapopulational variability; however, problems with the electrophoretic procedure, such as curved gel fronts (most likely caused by the grinding buffer and the pH of the stains) make any attempts at delivering quantifiable data for these photographed

gels impossible. There are two gels that have been scored for the enzyme PER (Tables 4 & 5). If one examines the $R_f$ values for the two Solitario populations (see Fig. 2) collectively (Table 4), it appears that Population #2 is more genetically variable for PER than is Population #1. Both populations appear to be somewhat homogeneous. Using Hartl and Clark (1989) as a guide, one might infer that there are two monomorphic PER loci ($R_f$ values of 0.04 and 0.15) and one polymorphic PER locus ($R_f$ values of 0.28 and 0.32) occurring in each of the Solitario populations. Because part of this gel was destroyed during staining, $R_f$ values from sample six to sample 16 are somewhat vague for the first enzyme band; however, it is believed that this locus is monomorphic. One might conclude that the polymorphic locus is monomeric, consisting of two alleles. Based upon this conclusion, the frequency of heterozygotes for the polymorphic locus in both populations is very low, calculated to be 0.125.

Electrophoretic banding patterns for the Shafter Population (Table 5) reveal that the genetic structure for one enzyme, PER, is completely different than the patterns recognized for the Solitario populations. Therefore, variability across the range of the Hinckley Oak is maintained, at least for one enzyme system. One might infer that there are three PER gene loci existing in the Shafter Population. The first locus ($R_f$ value 0.08) consists of one allele and is monomorphic. The second, polymorphic locus ($R_f$ values of 0.22 and 0.25) is considered to be monomeric, consisting of two alleles. Locus number three ($R_f$

values of 0.62, 0.65, and 0.71) consists of three alleles and is also polymorphic; however, it cannot be determined from these data whether the locus is monomeric or multimeric. For the second locus in the Shafter Population, the frequency of heterozygotes is calculated to be 0.75. Although variability at this polymorphic locus seems to be somewhat high, an overall view of the general PER electrophoretic banding patterns (three loci combined) reveals that Hinckley Oaks in the Shafter population are more similar in their PER genetic structure than they are different. The most notable difference for this population is seen in sample #2 which lacks one enzyme band that is common to the other three shrubs ($R_f$ value of 0.22). The fact that heterozygosity exists in the Shafter population is evidenced by the varying morphologies of growing seedlings whose acorns were collected from this population.

Although variability within the Solitario populations and the population in Shafter appears to be somewhat low, one must be careful not to jump to the conclusion that this variability is indicative of the variability that exists for all of the other enzyme systems. Further testing of several enzyme systems should better reveal the overall genetic structure of Hinckley Oak populations. Additionally, samples from Solitario and Shafter populations should be run on the same gel, in case the differences seen between these populations are not true differences but are mere artifacts of separate electrophoretic runs.

One small piece of evidence obtained in this study could possibly support the original hypothesis that variability within Hinckley Oak populations has always been somewhat low, regardless of hybridization. Putative hybrids were not found to exist within the Shafter Population nor was either variety of Q. pungens found to occur in or near this population, and yet variability for PER was still somewhat low. It is realized, however, that because of recent road construction in the Shafter area, Q. pungens trees might have been demolished. Therefore, thorough investigations concerning past and present distributions of these taxa in the Shafter area need to be initiated.

## FLAVONOID INVESTIGATIONS

Flavonoid analyses were more successful than the electrophoretic investigations and have provided an initial view of the overall population genetic structure of Q. hinckleyi populations. The techniques for flavonoid analysis are standard (Harborne, 1984) and have been carried out by Mr. Tomas Hernandez. Leaves and stems from small tip cuttings of Q. hinckleyi and Q. pungens var. pungens were taken to the laboratory where they were air-dried, ground in a Waring blender, and placed in a 75% aqueous methanol (MeOH) solution for extraction. Extraction occurred for 24 hours and the material was subsequently pressure-filtered through a Buchner funnel. Concentrated extracts were obtained by using a Buchler flash-evaporator, which removed the MeOH and left only water and the plant chemical compounds. The flavonoid constituents of this

concentrated extract were elucidated via two-dimensional descending paper chromatography. The solvents used were 3:1:1 (tert-butyl alcohol:acetic acid:water, v/v) and 15% glacial acid. Whatman number three chromatography paper was used.

The chromatograms, viewed under long-wavelength (366nm) ultra-violet light, initially revealed the presence of quercetin 3-0-diglycosides within the two taxa investigated (Hilsenbeck, 1990 Performance Report). Preliminary data suggest that Q. hinckleyi possesses 12 flavonoid compounds. Profiles generated for two Q. hinckleyi populations in the Solitario (see Fig. 2) reveal that 10 compounds are shared by Quercus pungens var. pungens and Q. hinckleyi. These compounds are labeled #1-6, #8-10, and #12 (Fig. 3 & 4). Compound #11 of Q. hinckleyi was not seen in any of the Q. pungens individuals sampled while Q. hinckleyi compound #7 showed up in two of the Q. pungens specimens. Initial interpretations of the chromatographic profiles reveal that intraspecific variability for Q. hinckleyi is greater between the two populations sampled than it is within these populations. To date, the flavonoid compounds are being isolated for identification. It is anticipated that, upon completion of his thesis, Mr. Thomas Hernandez will submit more comprehensive interpretations of the flavonoid studies.

## POLLEN ANALYSIS

Pollen grains of Q. hinckleyi and Q. pungens var. pungens were examined with Cotton Blue in Lactophenol in order to establish male fertility. A 90% pollen fertility of Q. hinckleyi

and a 95% pollen fertility of Q. pungens var. pungens were recognized. Based upon these initial data, the possibility of low reproductive rates resulting from male sterility in Hinckley oaks is doubtful (Hilsenbeck, 1990 Performance Report).

Pollen from herbarium specimens at Sul Ross State University of Q. pungens var. pungens and Q. hinckleyi was acetolyzed and mounted for scanning electron microscopy. Initial data reveal that there is a difference in the sexine layer of the pollen between the two species and in the size of the pollen grains (see fig. 3 of Weyerts, 1991 Performance Report). These data have been useful in determining putative hybridization between the species (see hybridization section of this report).

## MICROPROPAGATION

Micropropagation of Q. hinckleyi using small tip cuttings was performed by Ms. Hillary Loring. As previously reported (Hilsenbeck, 1990 Performance Report; Weyerts, 1991 Performance Report), micropropagation techniques were difficult to work out because: 1) explants in "sterile" culture were being contaminated and 2) young enough plants for successful propagation were not obtained. Micropropagation was therefore terminated early in the study. Further information regarding micropropagation techniques may be obtained from the principal investigator, Dr. Richard Hilsenbeck.

## SEED VIABILITY - SEEDLING MORPHOLOGY

A total of 148 acorns were collected (1 Sep 1991) from the

Shafter Population. The plants produced thousands of acorns during this good year. One hundred and twenty-seven Q. hinckleyi acorns were planted in plastic stubbies (13.5 cm long), while 21 were planted in one-gallon pots. Acorns in stubbies were planted in sterile potting medium containing a time-release fertilizer, Osmocote. The Osmocote was composed of 14 parts nitrogen, 14 parts phosphorus, and 14 parts potassium. Half of the acorns planted in stubbies were situated sideways in the potting medium, while the other half (plus one) were situated with the tip of the acorn facing down.

Eleven of the acorns planted in one-gallon pots were planted in sterile potting medium with Osmocote (14:14:14), hereinafter called Treatment #1, while 10 of the acorns were planted in soil that contained no time-release fertilizer but did contain limestone rocks at the base of the pot, hereinafter referred to as Treatment #2. All of the acorns planted in one-gallon pots were situated in the same direction (acorn tip down).

Seedling emergence dates along with the direction in which newly emerged seedlings had been planted (acorn tip down or sideways) were recorded. Weekly tallies for these data were kept. After several leaves had formed on a seedling, morphological aspects such as leaf size, adaxial and abaxial pubescence, and leaf spination were recorded.

Out of the 148 Hinckley Oak acorns that were planted, a total of 129 acorns germinated, yielding an overall 87% germination rate. Only one insect larva was seen to emerge from

the acorns that were planted, suggesting that insect larvae do not appear to be adversely affecting germination in Q. hinckleyi, at least in some years. In stubbies, the first Q. hinckleyi seedling to emerge did so 13 days after the acorns had been planted. Thus, beginning with day 13, weekly tallies were kept for seedling emergence. The tallies were collected over an 11-week period, the end of which appears to have marked the cessation of germination. Tallies (calculated in percents) for weekly germination are illustrated in Figure 5. Combined weekly totals indicate that 112 out of 127 Hinckley Oak acorns planted in stubbies germinated, yielding 88% germination. Of the acorns that germinated, 58 were planted with the acorn tip down and 54 were planted sideways, thus indicating that the direction in which an acorn gets situated in the soil (if it is down or sideways) has no affect on germination.

In the gallon pots, all of the acorns planted in Treatment #1 germinated while only six of the 10 acorns planted in Treatment #2 germinated. In Treatment #1, the first acorn to germinate did so 18 days after the acorns had been planted. The first visible seedling in Treatment #2 was noticed 24 days after those acorns had been planted. Because of low sample sizes for Treatments #1 and #2, weekly tallies of germination rates are not herein reported.

Because gallon pot germination rates for Treatment #2 were 40% lower than they were for Treatment #1, it might be inferred that optimal germination is dependent on the type or porosity of

the substrate present. Because the sample sizes were so small for the gallon pot treatments, however, conclusions about substrate effect(s) are tentative at this time.

To date, 100 year-old seedlings are thriving at Sul Ross State University. Eighty-two of these seedlings are in one-gallon pots at the SRSU greenhouse and 18 seedlings (the healthiest of the 100 seedlings) have been planted at the Sul Ross State University research field plot where they will continue to be studied.

Initial observations reveal that the leaves of the greenhouse seedlings are larger (ca. 5 mm longer and wider) than are the leaves of adult Hinckley Oak plants found in natural habitats. The leaf outline of the seedlings appears to be more ovate-elliptic while the outline of native Hinckley Oak adults is generally subrotund to subovate. There are generally 2-5 spinose tips on either margin of the seedling leaf blades, which is comparable to the 3-4 spinose tips present on adult leaves. The microscopic abaxial papillae characteristic of adult Q. hinckleyi leaves are present on the seedlings, but they are not very well-developed in the seedlings.

Although native Q. hinckleyi adults have leaves that are glabrous, leaves of most of the older Hinckley Oak seedlings examined exhibited a few small white trichomes along the cartilaginous margins and along the veins (mostly the midvein). Because some of the youngest seedlings possess these trichomes along the blade, I hypothesize that the few trichomes found on

the leaves of Hinckley Oak seedlings are eventually lost as the plants mature.

In addition to the seedlings growing in the greenhouse, seven apparent Q. hinckleyi seedlings have been observed near Q. hinckleyi plants in the Shafter Population (Weyerts, 1992 Performance Report). Never before have Hinckley Oak seedlings been observed in natural conditions (Kennedy and Poole, 1992). The presence of these natural apparent seedlings suggests expansion or rejuvenation of Hinckley Oak populations. Six of the apparent seedlings are found near the adult plants but are not directly underneath them. It is possible that these six apparent seedlings are young rhizome offshoots produced by the adult plants. Because the apparent seedlings are very comparable in external morphology to the seedlings growing in the greenhouse, I believe that these six juvenile plants are probably not rhizome offshoots of adult plants. The seventh juvenile plant is found underneath the periphery of an adult Hinckley Oak shrub. Because of its proximity and striking similarity to the shrub, I believe that it is possible that this juvenile is a rhizome offshoot of the mother plant. Only extensive digging, an undesirable approach, would provide a reliable answer.

## PUTATIVE HYBRIDIZATION WITH OTHER OAK TAXA

The presence of other oak species within and nearby the Solitario Q. hinckleyi populations, namely Q. pungens var. vaseyana and Q. pungens var. pungens, has been reported (Hilsenbeck, 1990 Performance Report; Weyerts, 1992 Performance

Report). One putative hybrid (Q. hinckleyi x Q. pungens var. pungens) is located at the upper limits of Q. hinckleyi Population #1 while another putative hybrid occurs in the middle of the drainage of Population #2 (see Fig. 2). In addition to these putative hybrids, there are five other putative hybrids from which specimens have been collected (housed in the SRSC Herbarium). Several techniques have been used in an effort to establish the parentage of these putative hybrids. The results and some techniques are discussed in the sections to follow.

MORPHOLOGY WITH FOLIAR TRICHOME EMPHASIS--Quercus hinckleyi possesses small, spine-tipped coriaceous leaves that are not more than 15 mm in length (Muller, 1951; Correll & Johnston, 1970). These subrotund leaves are 2-3 toothed on each side and are blue-green on their upper (adaxial) surface. Adaxial and abaxial leaf surfaces of Q. hinckleyi are essentially glabrous and glaucous. However, numerous white solitary hairs, using Hardin's (1976) terminology, can be found on the leaf blades and cartilaginous leaf margins of this taxon when the leaves are observed with increasing magnifications. One of the most distinguishing foliar characters for Q. hinckleyi is the presence of microscopic epidermal papillae on the abaxial leaf surface (Muller, 1954).

The usually coriaceous leaf blades of Q. pungens var. vaseyana are up to 6 cm in length, which is 3 cm shorter than the reported upper leaf length limit for Q. pungens var. pungens (Muller, 1951; Correl & Johnston, 1970). The usually hard and stiff leaves of var. pungens are elliptic to oblong while the

leaves of var. vaseyana are narrowly lanceolate to usually oblong. Both varieties possess a lustrous upper leaf surface. Leaf margins of var. pungens are undulately crisped and sometimes revolute while the margins of var. vaseyana are more cartilaginously thickened and not revolute. Neither variety of Q. pungens possesses microscopic epidermal papillae on the abaxial leaf surface.

One of the most distinguishing features for oak species in general is the trichome complement present on the adaxial and abaxial leaf surfaces (Hardin, 1976; Hardin 1979). Not only does this trichome complement provide insight into phylogenetic relationships between species, but it is also extremely remunerative in documenting hybridization. As previously mentioned, the leaves (both adaxial and abaxial surfaces) of Q. hinckleyi are essentially glabrous. The adaxial trichomes of Q. pungens var. pungens are mostly multiradiate and stellate with an occasional solitary type (non-glandular and unbranched) that is usually found on the midvein. Abaxial trichomes of Q. pungens var. pungens are mostly stellate, fused stellate and multiradiate. Adaxial trichomes of var. vaseyana are by far less numerous than they are in var. pungens. In fact, some specimens of var. vaseyana appear to be devoid of any hairs and are thus essentially glabrous. The abaxial leaf surface of var. vaseyana is densely white pubescent, composed mostly of white stellate hairs. As well, occasional multiradiate hairs can also be found on the abaxial leaf surface.

Leaves of the putative hybrid collected from Solitario Population #2 (see Fig. 2) are virtually glabrous. The leaves of the putative hybrid collected in Population #1, however, are somewhat pubescent and contain mostly multiradiate trichomes (in addition to the very fine solitary trichomes characteristic of Q. hinckleyi leaves) on their adaxial surface along with multiradiate and some stellate trichomes on their abaxial leaf surface. Although the average leaf length for the putative hybrid collected from Solitario Population #1 does not exceed 15 mm, the putative hybrid collected from Solitario Population #2 possesses leaves with an average leaf length of 20.66 mm. The papillae on the abaxial leaf surface of both putative hybrids in Solitario Populations #1 and #2 are reduced. The leaves of both of these putative hybrids are not as strongly spinose as are the leaves of a "good" Hinckley Oak.

Two other Q. hinckleyi specimens housed in the Sul Ross State University Herbarium appear to be putative hybrids according to their foliar trichome complements. Both of these specimens were collected in the Solitario. One of these specimens appears to have multiradiate hairs on the adaxial leaf surface and golden stellate hairs on the abaxial surface. The other specimen appears to have some multiradiate trichomes on the adaxial leaf surface and some white stellate and fused stellate trichomes on the abaxial leaf surface. Neither of these two specimens possesses an average leaf length of over 15 mm. On both of these specimens, the abaxial papillae are reduced. The

leaves of these two putative hybrids are strongly spinose.

Although three other specimens housed in the Sul Ross State University Herbarium appear to be putative hybrids with regard to their external morphology (larger leaves, ca. 22mm in length), these specimens do not possess intermediate foliar trichome complements and they all have fully-developed abaxial papillae that are characteristic of Q. hinckleyi. These three individuals are believed to be morphological variants of Q. hinckleyi. All of the putative hybrids observed appear to be mature individuals.

FLAVONOID CHEMISTRY---Hilsenbeck (First Research Report, 1990) has reported suspected hybridization between Q. pungens var. pungens and Q. hinckleyi at least at one locality in the Solitario. Profiles generated by the three putative hybrids found in the "Jackie" Population (Hilsenbeck, 1990 Performance Report) indicated bona fide hybridization.

Each of the two putative hybrids (Q. hinckleyi x Q. pungens var. pungens) in Population #1 and Population #2 of the Solitario (see Fig. 2) possessed 11 compounds and lacked compound #5 of Q. hinckleyi and Q. pungens var. pungens (see Fig. 3-4). Because neither novel compounds nor additive compounds were observed in the profiles of these two putative hybrids, it is possible that these individuals are not hybrids but are morphological variants of Q. hinckleyi. Additional morphological variants might be discovered in the 12 other populations of Q. hinckleyi known to exist in the Solitario. Chemical analysis of additional morphological variants, should they exist, might contribute

toward further understanding of the origin of these plants.

ELECTROPHORETIC ANALYSIS--Because intermediate banding patterns for the two putative hybrids occurring in Solitario Populations #1 and #2 (mentioned above in Flavonoid analysis) were not recognized (Table 4, Samples 4 and 12), it is once again suspected that these individuals are not hybrids, but are morphological variants of Q. hinckleyi. Because of problems with the electrophoretic procedure, concrete data regarding the putative hybrids found in the "Jackie" Population (Hilsenbeck, 1990 Performance Report) cannot be presented.

STIGMA-POLLEN ANALYSIS--As previously reported (Weyerts, 1991 Performance Report), differences in pollen grain sizes and between the pollen sexine layer of Q. hinckleyi and Q. pungens var. pungens exist. Because of these differences, I was able to examine Q. hinckleyi stigmas with the JEOL JSM-35C Scanning Electron Microscope and, under the direction of Dr. Richard Hilsenbeck, count the number and type of pollen grains present on the stigmas. Twenty-nine stigmas collected in April of 1990 by Dr. Richard Hilsenbeck were placed on eight numbered stubs and were viewed with the SEM. Approximately 62% of the stigmas observed (18 stigmas) appeared to have no pollen grains present (Table 6). One or more Q. hinckleyi pollen grains were found on 34% of the stigmas (10 stigmas) while one stigma (3.4% of observed stigmas) contained two pollen grains from what appeared to be Q. pungens var. pungens. One stigma contained one pollen grain from what appeared to be a composite as well as a Q.

hinckleyi pollen grain. The largest number of pollen grains found on any stigma was four. A total of twenty-two Q. hinckleyi pollen grains were found, yielding an average of 0.76 grains of this species per stigma. Three pollen grains from other species were observed (.10 foreign grains/stigma). Nine of the Q. hinckleyi pollen grains found (41%) appeared to be germinating. While some of the pollen grains occurred dorsally on the stigma lobes, the majority of the grains found were situated on the broadened ventral apex of the stigma lobes.

Pollen tube growth rates depend on the types of pollen present on a stigma (Daizen et al., 1990) and investigators have shown that successful fertilization of donor pollen is dependent upon the different types of pollen present on a stigma (Cruzan, 1990). If pollination phenomena are the cause of any lowered reproductive success of Q. hinckleyi, one might expect to find an excess of foreign pollen on the stigmas and/or an extremely low number of Hinckley Oak pollen grains per stigma, or one might expect to find stigmas overcrowded with Hinckley Oak pollen which could lead to pollen competition.

Only two of the 25 pollen grains observed (8%) were those of another oak species. Because approximately 34% of the stigmas contained Q. hinckleyi pollen, and an average of 0.76 Hinckley Oak pollen grains per stigma was observed (although less than an average of one per stigma), it is doubtful that any low reproductive rates of this species are a result of an inadequate amount of wind pollen being received. The fact that there was

less than one pollen grain on average per stigma coupled with the large, three-lobed nature of the Q. hinckleyi stigmas leads one to believe that pollen competition on an overcrowded stigma is not occurring. The largest number of grains found on any stigma was four, which would by no means delineate an overcrowded stigma.

## DISCUSSION

Quercus hinckleyi is very limited in its distribution. It is known to occur only in the Solitario, in Shafter, and in the Sierra Rica Mountains of Mexico (R. Spellenberg and D. Riskind, pers. comm.). The limited distribution of Q. hinckleyi fits the pattern exhibited by some oak species that have undergone rapid speciation in an opportunistic response to increasing climatic shifts in the region (Axelrod, 1983). The Chihuahuan Desert is one vast region that has experienced such climatic shifts over the past 10,000 years (Van Devender and Worthington, 1977). Continued climatic shifts in this area will probably continue to promote rapid speciation of oaks.

In its restricted range, Q. hinckleyi appears to be producing viable seeds, as evidenced by an 88% germination rate for acorns grown under greenhouse conditions. Under favorable conditions, therefore, Hinckley Oak acorns have viable embryos that have an 88% potential to germinate.

Initial interpretations of electrophoretic and flavonoid data reveal that interpopulational variability for Q. hinckleyi is greater than intrapopulational variability. The presence of

apparent morphological variants in the populations sampled further suggests that variability across the range of Q. hinckleyi exists. Therefore, Q. hinckleyi is probably not as homogeneous a species as once was suspected.

Although putative introgression from Q. pungens var. pungens can be recognized through the trichome complements of some of the suspected hybrids, the low number of putative hybrids recognized compared to the large number of apparently "true" Hinckley Oaks present in populations sampled does not seem to indicate extensive hybridization within the populations. Pollen-stigma analyses further suggest that Q. hinckleyi is not experiencing extreme pollen contamination or competition from another oak species. With 90% pollen fertility, Q. hinckleyi is probably not experiencing low reproductive rates as a result of male sterility. I conclude that the primary factors involved in limiting Q. hinckleyi populations are the extreme environmental conditions in which the oak is found.

In their draft of Hinckley's Oak (Q. hinckleyi) Recovery Plan, Kennedy and Poole (1992) report that little is known about the population biology, population ecology, or habitat requirements of Q. hinckleyi. I might also add that little is known about the taxonomic relationships of Q. hinckleyi, a most severe handicap when trying to predict the necessary time and economic resources to be allocated for recovery.

## REMARKS

This project was initiated at Sul Ross State University in March, 1990 by Dr. Richard A. Hilsenbeck, the principal investigator. In July, 1991, Dr. Hilsenbeck abruptly resigned his position at Sul Ross and abandoned the project. At the time, several students were involved with the research project under the direction of Dr. Hilsenbeck: Ms. Hillary Loring (Micropropagation/1990-1991), Mr. Trinidad Cantu (Electrophoresis/ 1990), Mr. Luis Villareal, (Electrophoresis/ 1990), Mr. Tomas Hernandez (Flavonoid Analysis/1990-1992), and Ms. Sharon Weyerts (Electrophoresis/ 1990-1991 and Sep-Nov 1992). With Dr. Hilsenbeck as our major advisor, Mr. Tomas Hernandez and I decided to incorporate our particular aspects of the research into thesis projects. Upon Dr. Hilsenbeck's resignation, Mr. Hernandez continued with flavonoid investigations and was funded for his efforts. I discontinued the electrophoretic studies in September of 1991, for reasons already stated, and was not employed through Sul Ross State University with Texas Parks and Wildlife funds. Additionally, under the guidance of Dr. A. Michael Powell, my major advisor, my thesis project changed. Although still involving Q. hinckleyi, my thesis does not follow the same guidelines that were established by Dr. Hilsenbeck, essentially some of those set forth in the original Hinckley Oak contract. The last data sets being gathered, for which I am funded by the Texas Parks and Wildlife Department (Sep 1992 - Nov 1992) involve mainly SEM surveys of foliar trichomes exhibited by

Q. hinckleyi and four closely related taxa. These data, along with other data, will be presented in my thesis, which will be submitted to the Texas Parks and Wildlife Department upon its completion. Although my thesis will contain information regarding the biology of Q. hinckleyi, it should be considered a separate entity and not be construed as a follow-up to or a part of the final report. It is anticipated that more comprehensive analyses of flavonoid investigations will be submitted by Mr. Tomas Hernandez upon completion of his thesis. Additional information regarding this final report, if needed, might be obtained from the principal investigator, Dr. Richard A. Hilsenbeck (195 Teal Lane, Tallahassee, FL 32308).

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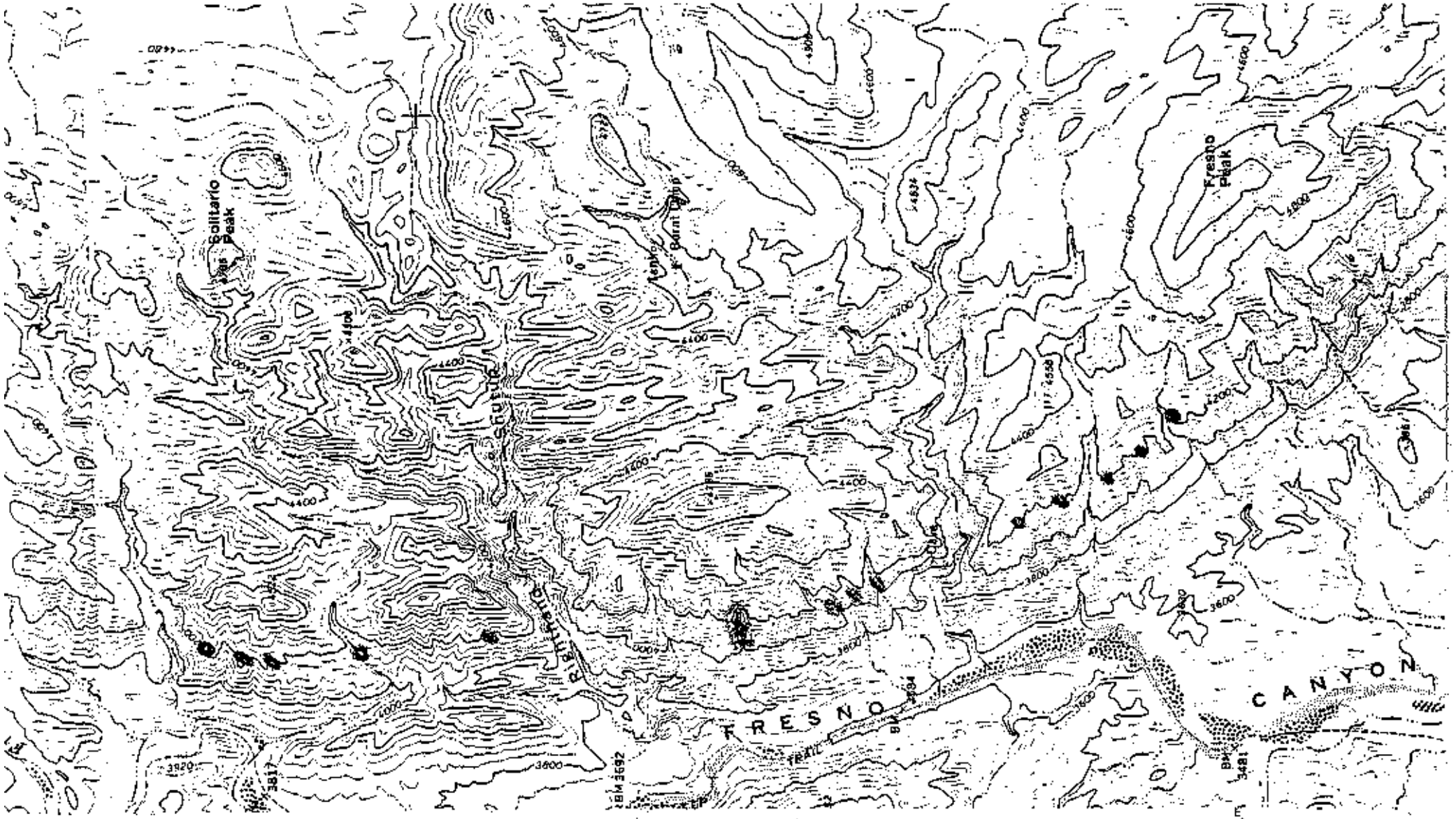


Figure 1. Locations of 14 O. hinckleyi populations in the Solitario.

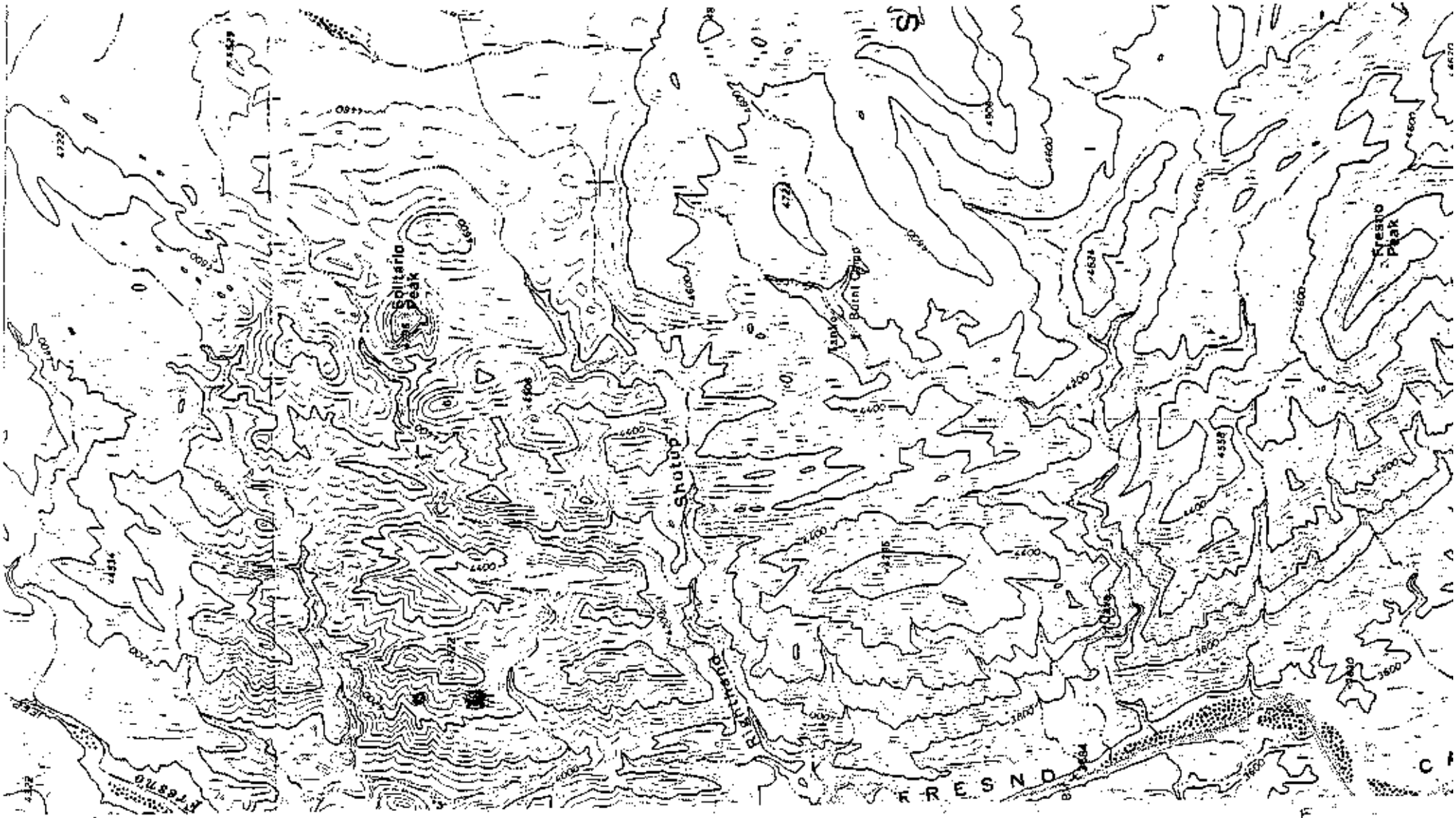


Figure 2. Locations of two Solitario populations sampled during the 1991-1992 research period.

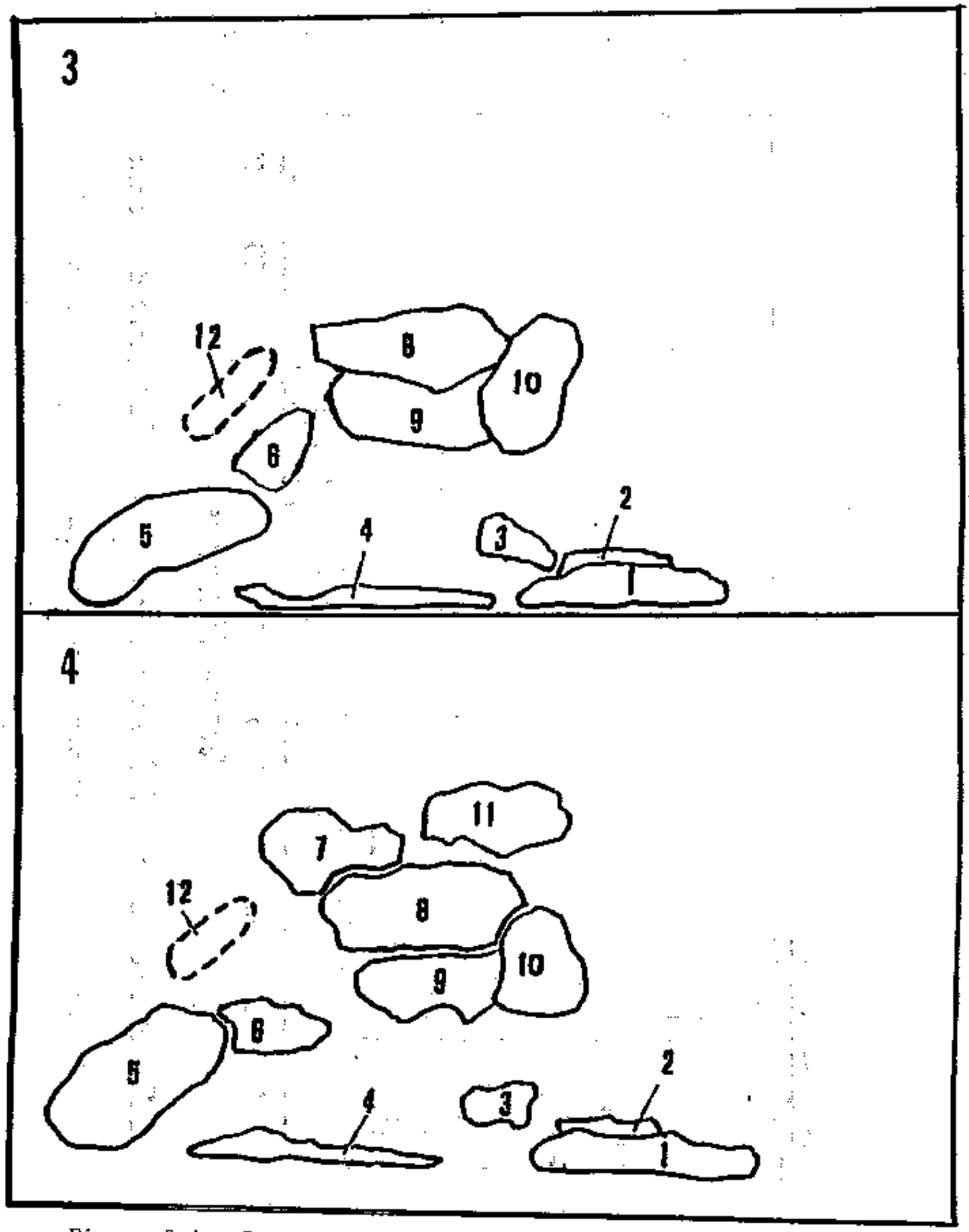


Figure 3-4. Representation of two-dimensional chromatograms generalized from two populations. 3. Q. pungens var. pungens. 4. Q. Hinckleyi.

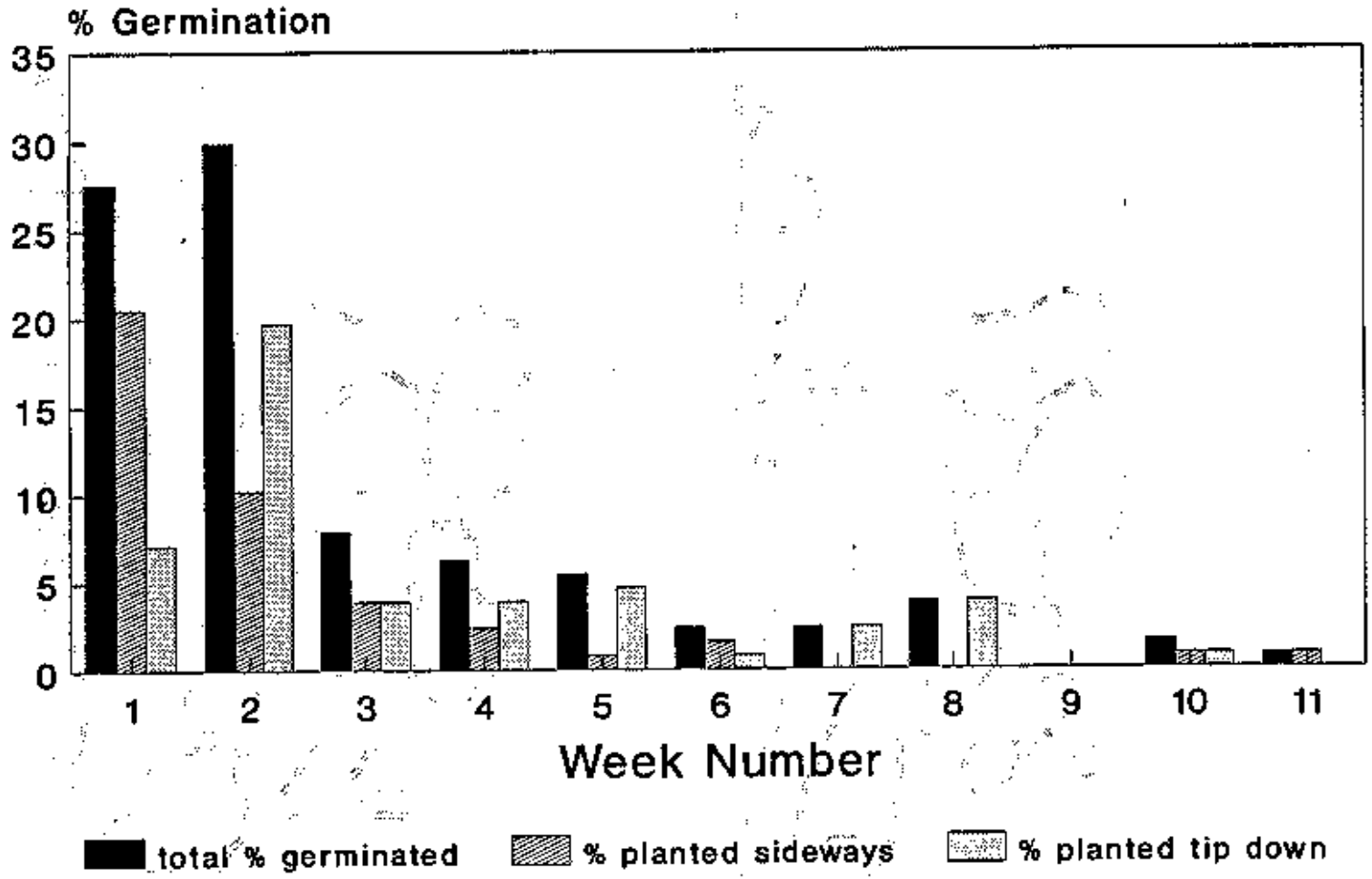


Figure 5. Tallies for weekly germination of Q. hinckleyi seedlings.

Table 1. Plant species occurring in Hinckley Oak Populations

| Species | Population | | |
|---|---|---|---|
| | #1 | #2 | Shafter |
| Acacia roemeriana | X | X | X |
| Agave lechuguilla | X | X | X |
| Ariocarpus fissuratus | X | X | |
| Aristida glauca | X | X | |
| Asclepias asperula | | | X |
| Ayenia pilosa | | | X |
| Berberis spp. | | | X |
| Bernardia obovata | X | X | X |
| Bouteloua curtipendula | X | X | X |
| Buddleja marrubiifolia | X | X | |
| Castilleja rigida | X | X | |
| Centaurium calycosum | X | X | |
| Chrysactinia mexicana | X | X | |
| Condalia ericoides | | | X |
| Coryphantha sneedii var. albicolumnaria | X | X | |
| Cowania ericifolia | X | X | |
| Croton pottsii | | | X |
| Croton sancti-lazari | X | X | X |
| Cuscuta spp. (parasitic on Zexmenia brevifolia) | | | X |
| Dalea aurea | | | X |
| Dalea formosa | X | X | |
| Dasylirion leiophyllum | X | X | X |
| Echinocereus dasyacanthus | X | X | |
| Encelia scaposa | | | X |
| Ephedra aspera | X | X | |
| Epithelantha bokei | X | X | |

Table 1. Continued.

| Species | Population | | |
|---|---|---|---|
| | #1 | #2 | Shafter |
| Erigeron modestus | X | X | |
| Eriogonom havardii | X | X | |
| Euphorbia antisyphilitica | X | X | |
| Fendlera rigida | X | X | |
| Fouquieria splendens | X | X | |
| Hechtia texensis | X | X | |
| Hedeoma nanum var. nanum | X | X | |
| Hedyotis acerosa | | | X |
| Hedyotis nigricans | X | X | |
| Heliotropium torreyi | | | X |
| Leucophyllum minus | X | X | X |
| Linum rupestre | X | X | X |
| Menodora longiflora | | | X |
| Menodora scabra | | | X |
| Mimosa biuncifera | | | X |
| Mortonia sempervirens subsp. scabrella | X | X | X |
| Muhlenbergia dubia | X | X | |
| Nerisyrenia camporum | | | X |
| Notholaena parvifolia | X | X | |
| Opuntia spp. | X | X | |
| Parthenium argentatum | X | X | |
| Parthenium incanum | | | X |
| Penstemon baccharifolius | X | X | |
| Petrophytum caespitosum | X | X | |
| Phyllanthus polygonoides | | | X |
| Poliomintha glabrescens | X | X | |

Table 1. Continued.

| Species | Population #1 | #2 | Shafter |
|---|---|---|---|
| Polygala macradenia | | | X |
| Porophyllum scoparium | | | X |
| Prosopis glandulosa | | | X |
| Rhus virens | X | X | |
| Ruellia parryi | X | X | X |
| Schizachyrium scoparium var. neomexicanum | X | X | |
| Selaginella lepidophylla | X | X | X |
| Stenandrium barbatum | | | X |
| Thelesperma longipes | X | X | |
| Thelesperma megapotamicum | | | X |
| Thymophylla acerosa | | | X |
| Tridens muticus | X | X | |
| Viguiera stenoloba | | | X |
| Yucca thompsoniana | X | X | |
| Zexmenia brevifolia | X | X | X |

Table 2. Buffer systems and their components that were used for electrophoretic analysis of Quercus hinckleyi[a]

| Buffer System | pH | Electrode Buffer | Gel Buffer |
|---|---|---|---|
| Histidine-Citrate | 5.7 | L-Histidine Free Base | Electrode Buffer |
| | | Citric Acid | Distilled Water |
| Lithium-Borate | 8.3 | Lithium Hydroxide | Tris |
| | | Boric Acid | Citric Acid |
| | | Distilled Water | Electrode Buffer |
| | | | Distilled Water |
| Histidine-Citrate | 7.0 | Citric Acid | Histidine-HCl |
| | | Distilled Water | Distilled Water |

[a]Buffer systems used were those of Soltis et al., 1983.

Table 3. Enzymatic stains corresponding to three buffer systems used

| Buffer System | pH | Enzyme Stains |
|---|---|---|
| Histidine-Citrate | 7.0 | IDH, PGD, SKDH |
| Lithium-Borate | 8.3 | PGI, GOT, LAP, TPI, GDH |
| Histidine-Citrate | 5.7 | PER, MDH, ADH, PGM |

Table 4. Peroxidase $R_f$ values for sixteen Hinckley Oaks occurring in the Solitario

| Population #1 Samples | | | | | | | | Population #2 Samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4* | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12* | 13 | 14 | 15 | 16 |
| 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| 0.28 | | | | | | | | | 0.28 | 0.28 | 0.28 | 0.28 | | | |
| 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | 0.32 | | | | | 0.32 | 0.32 | 0.32 | 0.32 |

*Putative hybrids.

Table 5. Peroxidase $R_f$ values for four Hinckley Oaks in the Shafter Population

| Sample No. | | | |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| 0.08 | 0.08 | 0.08 | 0.08 |
| 0.22 | | 0.22 | 0.22 |
| 0.25 | 0.25 | 0.25 | 0.25 |
| | | 0.62 | |
| | | | 0.65 |
| 0.71 | | | |

Table 6. Number and type of pollen grains present on Q. hinckleyi stigmas

| Stigma No. | Number of Grains | | | |
|---|---|---|---|---|
| | Hinckley Oak[a] | Other | Hinckley Oak[b] | Total |
| 1 | 0 | 2 | 0 | 2 |
| 2 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 4 | 4 |
| 4 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 2 | 2 |
| 6 | 1 | 0 | 1 | 2 |
| 7 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 0 | 0 |
| 9 | 2 | 0 | 0 | 2 |
| 10 | 0 | 0 | 0 | 0 |
| 11 | 0 | 0 | 0 | 0 |
| 12 | 0 | 0 | 0 | 0 |
| 13 | 0 | 0 | 0 | 0 |
| 14 | 2 | 0 | 0 | 2 |
| 15 | 0 | 0 | 2 | 2 |
| 16 | 0 | 0 | 0 | 0 |
| 17 | 0 | 1 | 1 | 2 |
| 18 | 0 | 0 | 0 | 0 |
| 19 | 0 | 0 | 0 | 0 |
| 20 | 1 | 0 | 0 | 1 |
| 21 | 0 | 0 | 0 | 0 |
| 22 | 0 | 0 | 0 | 0 |

Table 6. continued.

| Stigma No. | Number of Grains | | | |
|---|---|---|---|---|
| | Hinckley Oak[a] | Other | Hinckley Oak[b] | Total |
| 23 | 0 | 0 | 0 | 0 |
| 24 | 2 | 0 | 2 | 4 |
| 25 | 1 | 0 | 1 | 2 |
| 26 | 0 | 0 | 0 | 0 |
| 27 | 0 | 0 | 0 | 0 |
| 28 | 0 | 0 | 0 | 0 |
| 29 | 0 | 0 | 0 | 0 |

[a]Number of grains that appeared to be germinating

[b]Number of grains that weren't germinating