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DISSERTATION

BIOSYSTEMATIC STUDIES OF OENOTHERA L. SUBGENUS PACHYLOPHUS

Submitted by

Robert Erland Stockhouse II

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

August, 1973

COLORADO STATE UNIVERSITY

August, 1973

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ROBERT ERLAND STOCKHOUSE II ENTITLED BIOSYSTEMATIC STUDIES OF OENOTHERA L. SUBGENUS PACHYLOPHUS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

J. W. Brewer

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ABSTRACT OF THESIS

BIOSYSTEMATIC STUDIES OF OENOTHERA L.

SUBGENUS PACHYLOPHUS

Evolutionary relationships of the ten species of Oenothera subgenus Pachylophus were established on the basis of data from studies of morphology, cytology, distributions, habitat requirements and pollination ecology.

It was found that the seven subspecies of Oenothera caespitosa Nutt., Oe. eximia A. Gray and Oe. psammophila (Nels. & Macbr.) Stockhouse, form a closely related alliance. Ecogeographic differentiation and pollinator preferences have promoted evolution within this alliance. Chromosomal restructuring was minimal in the establishment of these taxa. Oenothera caespitosa Nutt. subsp. eximia (A. Gray) Munz and subsp. montana (Nutt.) Munz var. psammophila (Nels. & Macbr.) Munz were elevated to the specific level; Oenothera eximia A. Gray and Oenothera psammophila (Nels. & Macbr.) Stockhouse.

Oenothera cavernae Munz and Oe. brandegeei (Munz) Raven have differentiated recently from Oe. caespitosa through habitat specialization, changes to the annual growth habit and a change from allogamy to autogamy.

Oenothera tubifera Seringe in DC Prodr. and Oe. muelleri Munz were more distantly related to the above alliance through Oe. macrosceles A. Gray. Oenothera tubifera was probably of recent origin from muelleri.

Oenothera primiveris A. Gray and Oe. xylocarpa Coville were shown to be more distantly related to the Oe. caespitosa alliance. Oenothera xylocarpa appears to have originated from Oe. primiveris.

Evolution within the subgenus Pachylophus has occurred within the framework of minimal chromosomal restructuring, especially when compared with the more advanced subgenera of Oenothera.

Pollination ecology studies of Oenothera caespitosa and Oe. psammophila indicated that the two were ethologically isolated by hawkmoth pollinator preferences. Intra and interpopulation gene flow were studied. Interpopulation hawkmoth flights in excess of twenty miles were documented. It was suggested that the polytypic nature of many Oe. caespitosa populations may be due to hawkmoth mediated gene flow between populations. It was also shown that intersubspecific gene flow was probably occurring.

Individual flowers of Oenothera caespitosa produced on the average, 35 microliters of nectar. The sugar concentration of the nectar was found to be approximately 32%. It was calculated that approximately 42 calories of energy were available in each caespitosa flower for

hawkmoth consumption. It was also shown that hawkmoths may be deriving essential amino acids from nectar pollen mixtures occurring in the hypanthia of Oe. caespitosa flowers.

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INTRODUCTION

Oenothera L. (Onagraceae) is a genus of approximately 80 species. The genus is widespread in temperate and subtropical North and South America, but most diverse in the southwestern United States and adjacent Mexico. Oenothera is divided into ten subgenera which clearly represent variations on a common theme (Raven, 1964b).

Most of the cytogenetic and phylogenetic research for which Oenothera is well known has been on the North American representatives of the subgenus Oenothera (Hagen, 1950; Cleland, 1972). (Subgenus Oenothera is commonly referred to in the literature as Onagra or Euoenothera). Plants of this subgenus have evolved rapidly to their present state in spite of a number of potentially deleterious features of their breeding system. Their breeding system incorporates reciprocal translocations through which linkage groups have been developed and genetic recombination reduced; an accumulation of lethals which ensure structural heterozygosity; and self-pollination, which keeps the complex genetic mechanism intact. Any one of these processes by itself would be damaging, but together, they have combined to form a very successful genetic system. According to Cleland (1972) members of the subgenus Oenothera have substituted present adaptedness for the possibility of future evolutionary development and in this respect are

representatives of a very specialized genetic system. (For a discussion of the classical Oenthera system see: Blakeslee and Cleland, 1930; Cleland and Blakeslee, 1930; Cleland, 1931, 1935, 1936, 1957, 1962, 1972; Burnham, 1962).

Other subgenera of Oenothera have been studied less. Hecht (1944, 1950) and Cleland (1968) have worked on cytogenetic and phylogenetic relationships of subgenus Raimannia. Klein (1964, 1970) investigated the biosystematic relationships among the members of subgenus Anogra. Parnell (1969) looked at the South American subgenus Hartmannia. Other studies have dealt with various characteristics of one or more subgenera (Emerson, 1938, 1939; Steiner, 1956, 1964; Gregory and Klein, 1960; Hecht, 1960; Lewis and Raven, 1958; Laws, 1965, 1967; Kumar and Hecht, 1967; Khafaja and Steiner, 1970; Zinmeister and Bartl, 1971; etc.).

Previous taxonomic studies of subgenus Pachylophus have primarily utilized external morphology and have been descriptive in nature. In 1931, Munz included four species in the subgenus Pachylophus. These species were Oenothera caespitosa Nutt., the type species Oe. primiveris Gray; Oe. tubifera Seringe in DC Prodr.; and Oe. xylocarpa Coville. Munz recognized 11 infraspecific taxa of Oe. caespitosa at the varietal level which exhibited great morphological variation.

In 1941, Munz described Oenothera cavernae, a new species, and placed it in the subgenus Pachylophus.

Munz (1965), in a more recent monograph, recognized the above five species within the subgenus Pachylophus. However, the number of infraspecific taxa of Oe. caespitosa was reduced to 9 by merging var. longiflora into var. marginata and var. psammophila into var. montana. These nine infraspecific taxa were then elevated to subspecific status. Munz also partitioned the variation within Oe. primiveris into 3 subspecies at this time. The relationships within Pachylophus, however, remained provisional as he states "so little is known about the cytogenetics of this complex (Oe. caespitosa) that only a tentative presentation can be given of the taxa involved" (Munz, 1965).

Goeken (1969) studied four subspecies of Oenothera caespitosa: subspecies crinita; eximia; marginata; and montana. Morphological and cytological affinities were reported among these four subspecies which supported the taxonomic treatment of Munz (1965), and it was recommended that the four subspecies remain conspecific. Goeken's study, however, was based on limited material and little cytological or breeding data.

In 1970, Raven redefined the limits of Pachylophus to include 8 species. He recognized the 5 species of Munz (1965) but, raised Oe. caespitosa subsp. brandegeei to the specific level on the basis of morphological evidence. Oe. macrosceles A. Gray and Oe. muelleri Munz, previously included in subgenus Raimannia (Munz, 1935, 1965), were removed and added to Pachylophus. Both morphological and cytological

evidence (Cleland, 1968) were cited for this change. Raven (1970) in reviewing the other species of Pachylophus, pointed to the rather uncertain affinities within the subgenus. He states that "relationships within this group (Pachylophus) need further clarification by biosystematic studies" (Raven, 1970).

The present study was undertaken to provide a more complete understanding of the biosystematic relationships within Pachylophus. Data were gathered from morphological, cytological, breeding and pollination ecology studies. Species distributions and habitats were also studied.

Few chromosome counts have been published for the taxa within the subgenus Pachylophus. Gregory and Klein (1960) investigated a single population of Oe. caespitosa subsp. marginata and found a gametic number of n = 7 and a meiotic association of five bivalents (5II) plus a chain of four chromosomes. Kurabayashi, et al. (1962) reported a single count for marginata (n = 7). Geoken (1969) cites eleven additional counts: a tetraploid count for Oe. caespitosa subsp. crinita (n = 14; 5IV + 4II); four diploid counts of subsp. marginata (7II); five counts for subsp. montana (4 diploids of 7II, and one tetraploid, 5IV + 4II); and a diploid count for Oe. eximia (7II).

Gregory and Klein (1960) reported n = 7 for Oe. xylocarpa. Raven (herbarium sheep, RSA, Raven 14263) reported a meiotic configuration of 2θ4 + 3II for xylocarpa. Hecht (1950) investigated Oe. macrosceles

and found seven bivalents. Cleland (1968) reports 5II + 04 for macrosceles. Klein (herbarium sheet, RSA, Gregory 380) reported 7II for Oe. primiveris. There are no chromosomal data in the literature for Oe. brandegeei, cavernae, muelleri, psammophila, or tubifera.

During the course of this study it became apparent that the pollination ecology of Oe. casepitosa was important to the understanding of the biosystematic relationships of the taxa within Pachylophus. A part of two summers was spent studying the pollination ecology of Oe. caespitosa.

Pollination ecology has been the subject of numerous recent publications (Linsley, 1958; Linsley and MacSwain, 1958; Macior, 1965, 1966, 1967, 1968, 1970, 1971; Baker, 1961; Meeuse, 1961; Grant, 1952; Grant and Grant, 1964, 1965; Faegri and van der Pijl, 1966; Heinrich and Raven, 1972; Levin, 1971; etc.). Baker (1961) discussed the adaptation of flowering plants to nocturnal and crepuscular pollinators and the importance of various insurance mechanisms in maintaining the species which are moth pollinated. Heinrich and Raven (1972) pointed out the importance of the energy budget of pollinators in relation to the food provided by the flowers they visit and discussed the role that this energy budget played in the evolution of flowering plants. Coevolutionary relationships between plants and animals have been treated by Janzen (1966, 1971), van der Pijl and Dodson (1966), Dodson (1962,

1970), Dodson, et al. (1969), Ehrlich and Raven (1969), Gilbert (1971, 1972) Levin (1971), etc.

Gregory (1963-64) published a comprehensive treatment of hawkmoth pollination of Oenothera. This treatise dealt primarily with Oe. hookeri (subg. Oenothera). but seven other subgenera, including Pachylophus, were considered. The study compared the breeding systems of species from the following subgenera: Anogra; Gauropsis; Lavauxia; Megapterium; Oenothera; Pachylophus; Raimannia; and Salpingia. Six populations of Oe. caespitosa were investigated, but the observations were made at each population site on only one night. Gregory states that "more work on a species such as Oe. caespitosa is needed . . . before positive conclusions can be drawn."

Floral constancy of hawkmoths has not been extensively documented (Lovell, 1918; Gregory, 1963-64; Janzen, 1971). Gregory (1963-64) reported that Hyles frequents Oenothera in the deserts of the southwest, but, that they are not always constant to Oenothera when alternate nectar sources are nearby. He observed Hyles visiting Cirsium vulgare when Oe. strigosa flowers were less than one foot away.

Hawkmoth biology and pollination ecology has been studied recently by several other investigators (Baker, 1961, 1963; Meeuse, 1961; Heinrich, 1971, 1972; Heinrich and Bartholomew, 1971) but inconsistencies dealing with Oenothera have arisen. For instance Faegri and

van der Pijl (1966) state that "the flowers of Pachylobus caespitosus (sic) (Oe. caespitosa) . . . are visited by (hawk) moths. However, when the blossoms open, anthers dehiscing and stigma receptive, these organs are covered with very small black dipters. That these animals must at least cause self-pollination seems obvious."

Statements such as the above led to the present investigation of the pollination ecology of Oe. caespitosa, since Oe. caespitosa has long been known to be self-incompatible (East, 1940; Hagen, 1950) and black dipters obviously do not cause self-fertilization.

Subgenus Pachylophus, as treated in the present study, is composed of ten species. In addition to the eight species recognized by Raven (1970), biosystematic evidence provided by this thesis has shown that Oe. caespitosa subsp. eximia and Oe. caespitosa subsp. montana var. psammophila should be elevated to specific status.

Oe. brandegeei (Munz) Raven, Oe. caespitosa Nutt., Oe. eximia A. Gray, and Oe. psammophila (Nels. and Macbr.) Stockhouse were studied in detail. Oe. macrosceles A. Gray, Oe. muelleri Munz, Oe. primiveris A. Gray, and Oe. tubifera Seringe in DC Prod. were treated in a less comprehensive manner because of the limited materials available. Only herbarium species of Oe. cavernae Munz and Oe. xylocarpa Coville were examined.

MATERIALS AND METHODS

Plants from populations of four species of Oenothera, subgenus Pachylophus, were collected during 1969-1972 from the field and propagated in a greenhouse at Colorado State University. This material covered an extensive portion of the ranges of Oe. caespitosa, Oe. eximia and Oe. psammophila. Limited material was obtained of Oe. primiveris. Seeds of Oe. tubifera, Oe. muelleri, Oe. macrosceles and Oe. brandegeei were obtained from P. H. Raven (Missouri Botanical Garden, St. Louis, Mo.) and W. Stubbe (Botanisches Institut der Universitat, Dusseldorf).

Distributional and morphological data were obtained from the following herbaria which are designated according to the standard abbreviations of Lanjouw and Stafleu (1954): Colorado State University (CS). Ft. Collins, Colorado; Pomona College (POM), Claremont, California; Rancho Santa Ana Botanic Garden (RSA), Claremont, California; Southern Methodist University (SMU), Dallas, Texas; University of Arizona (ARIZ), Tucson, Arizona; University of Colorado (COLO), Boulder, Colorado; University of Indiana (IND), Bloomington, Indiana; University of Minnesota (MIN), Minneapolis, Minnesota; University of New Mexico (NMU), Albuquerque, New Mexico; University of Texas (TEX), Austin, Texas; University of Wyoming (RM), Laramie, Wyoming;

United States Forest Service Herbarium (USFS), Ft. Collins, Colorado; United States National Herbarium (US), Washington, D. C.

Personally collected material, greenhouse propagations and specimens from the above herbaria were used for morphological studies. Voucher specimens were deposited in the Colorado State University Herbarium (CS) in Ft. Collins, Colorado.

Habitat information was gathered in the field. Soil samples from many of the collection sites were analyzed by the Boyoucous hydrometer method to determine textural grade. Temperature and precipitation data were obtained from climatic summaries prepared by the Weather Bureau for the different sites.

Buds for meiotic chromosome studies were collected from field populations or propagation material in the greenhouse. They were fixed in glacial acetic acid and absolute ethanol (1:3, v/v) for 3-24 hours. Then they were changed to 70% ethanol and kept under refrigeration. Prior to staining, the buds were hydrolysed in 1:1 concentrated HCl and 95% ethanol for 8-11 minutes (Lewis and Lewis, 1955). The anthers were squashed in acetocarmine, and the slides were made permanent through the addition of Hoyer's mounting medium following the procedure described by Beeks (1955). Subsequent observations were made with a Zeiss Standard WL microscope with phase contrast or a Zeiss Photomicroscope II equipped for Normarski differential

interference contrast. Meiotic configurations were documented with camera lucida drawings or photomicrographs.

Breeding systems were studied by direct observation in the field and in the greenhouse. Compatibility was determined for at least three plants from each collection site and from greenhouse materials. Plants were emasculated, selfed, and put in insectproof cages for 48 hours. Styles were collected after 24-48 hours and placed in a saturated solution of potassium iodide. Pollen tubes in Oenothera are lined with starch grains which accept the iodine, and can be seen in the style as dark, granular beads or threads (Renner, 1919). Styles were examined for pollen tube penetration and measurements of penetration were made. Plants that did not set seed and/or the pollen tube failed to penetrate to the base of the style were classified as self-incompatible. Plants were classified as self-compatible if seed was set in the capsule.

Plants to be crossed were isolated in an insect-proof cage, emasculated, and the desired crosses performed. To guard against pollen contamination, plants were kept in an insect-proof cage until the flowers withered.

Per cent stainable pollen for both the parental material and the F_1 hybrids was calculated. Pollen was stained with cotton blue in a solution of lactophenol. Acceptance of stain was assumed to indicate viability (Raven, 1962; Thien, 1969). Per cent stainable F_1 pollen

was used in conjunction with chromosome pairing to indicate the ability of two parental genomes to form fertile progeny.

Damage by insects, crown rot, and bud blasting in greenhouse plants limited the material available for cytological study. Inadvertent fogging of the greenhouse in 1971 with a herbicide-like compound resulted in extensive losses and severely limited the scope of the F_1 hybrid study. Approximately 75% of the parental stock and all of the F_1's in propagation were lost.

The importance of hawkmoths and other insects as potential pollinators of the allogamous species of Pachylophus was recognized by Gregory (1963-64). Because of the importance of the seemingly obligate relationships of members of Oe. caespitosa to hawkmoth pollen vectors, a portion of two summers was spent analyzing the pollination ecology of Oe. caespitosa.

Intra- and interpopulation gene flow, hawkmoth/plant specificities and hawkmoth floral constancy were studied. The rate of turnover, i.e., the number of hawkmoths entering and leaving Oenothera caespitosa populations per night, was also studied.

Intrapopulation pollination was examined at several populations of Oe. caespitosa in Colorado and Utah and at populations of Oe. psammophila in Idaho. At each site the population was divided into several sections and the flowers, and pollen in each section were dusted with a different color of micronized fluorescent dusts before

sunset (Stern and Mueller, 1968). Hawkmoths visited the populations during the night, unmolested. The flowers were collected the following morning and analyzed under ultraviolet light for the presence and quantity of the different colors of dust and pollen. Both quantitative and qualitative measurements of pollen transfer (gene flow) within these populations were determined by observation.

Interpopulation gene flow was studied by marking populations with different colors of micronized dust. The flowers from these and other populations in the area were collected the following mornings and analyzed under ultraviolet light for foreign colors.

Field observations were made in Utah, Colorado and Idaho to determine the rate of turnover of individual hawkmoths in the local hawkmoth populations. Moths were netted on successive nights, marked with fluorescent dust or latex paint, and released. Marked moths recaptured on successive or later nights were recorded. A black-light insect trap was also used to capture and attract moths for this study.

Insects were identified by R. W. Hodges, Systematic Entomology Laboratory, G. E. Bohart, Bee Biology and Systematics Laboratory, U. S. D. A. and J. W. Brewer, Department of Zoology and Entomology, Colorado State University.

Oenothera flowers were analyzed for the presence of ultraviolet reflecting patterns. The flowers were photographed under ultraviolet light with a 35 mm camera equipped with a Kodak Wratten 18A ultraviolet

transmitting filter. Kodak Panatomic-X film was used because of its wide latitude in the ultraviolet range.

The amount of nectar produced by individual Oenothera flowers was measured. Nectar was collected in capillary tubes (5 microliter) between 8 pm and 8 am at 4 hour intervals.

The component sugars of nectar from eight Oenothera species were determined using paper chromatography. Whatman No. 1 filter paper (5" x 18") was spotted with 5 microliters of nectar from each species and standard sugars. Each chromatogram was run in butanol, ethanol and water (BEW) 10:6:4 v/v/v for 44 hours until the solvent had nearly reached the end of the paper. The chromatograms were allowed to dry and were sprayed with a sugar spray (80 ml 95% ethanol, 10 ml of 40% TCA and 10 ml of glacial acetic acid which was saturated with benzidine dihydrochloride) which made the sugars visible.

Gilbert (1972) reported that butterflies of the genus Heliconius feed on pollen by removing amino acids and proteins from it. These amino acids undoubtedly play an important role in the reproductive and population biology of these insects and Gilbert suggests that "other animals may use pollen in a similar fashion".

During the course of this study it was noted that most hypanthia collected in the field contained appreciable amounts of pollen. Therefore nectar and pollen were analyzed for amino acids using paper chromatography. Pollen was added to the hypanthia of greenhouse

plants and after 30 minutes, 5 microliters of the nectar solution spotted on Whatman No. 1 filter paper. The chromatograms were run for 44 hours in BEW then dried and analyzed for the presence of amino acids with ninhydrin. Amino acid standards were co-chromatogrammed to aid in the tentative identification of unknown amino acids.

The percentage sucrose in nectar samples of several taxa of the subgenus Pachylophus was determined using a Bausch & Lomb low range hand refractometer.

RESULTS AND DISCUSSION

DISTRIBUTIONAL AND ECOLOGICAL STUDIES

Species of Oenothera, subgenus Pachylophus, are found over a wide geographic range. Species distributions are shown in Figs. 1 and 2.

Oenothera caespitosa Nutt., the type species of the subgenus, is found in western North America from northern Mexico to southern Canada. The seven subspecies of Oe. caespitosa are sympatric over wide areas and occupy diverse habitats throughout the Great Basin and Rocky Mountains (Fig. 2).

Temperature and precipitation data for the seven subspecies of Oe. caespitosa are summarized in Figs. 3-7. Average elevations of known populations and average soil textures for habitats of five of the seven subspecies are presented in Table I. Habitat photographs are presented as Figs. 8-12.

Data from the above clearly shows that the subspecies of Oenothera caespitosa are adapted to a wide range of environments. Ecogeographic isolation seems to be apparent among subspecies.

Oenothera psammophila is endemic to the sand dunes west of St. Anthony, Idaho, an area of several square miles. It is found on open

Figure 1. Geographic distribution of the ten species of subgenus Pachylophus.

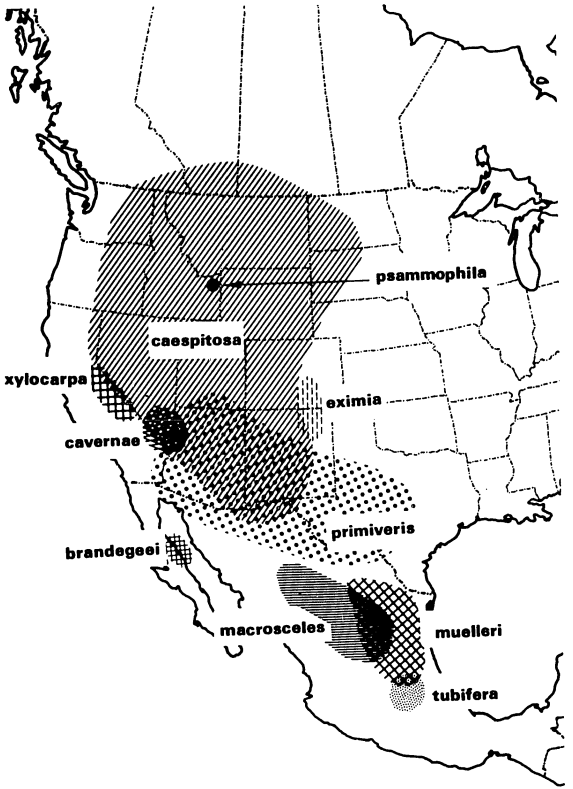
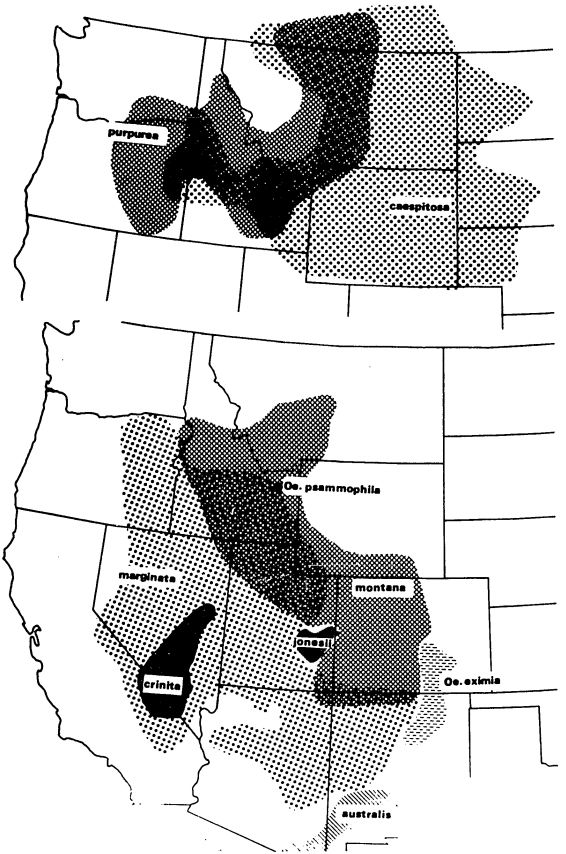


Figure 2. Geographic distribution of the seven subspecies of Oenothera caespitosa, Oe. eximia and Oe. psammophila.



Figures 3-7. Summary of climatic data for Oenothera caespitosa Oe. eximia and Oe. psammophila. Data taken from recording stations near known populations of the taxa. Based upon 24-34 year averages compiled form the Climates of the States, U. S. Dept. Commerce, Weather Bureau.

Figure 3. Graph of mean annual temperature and precipitation for Oe. caespitosa and Oe. eximia.

Figure 4. Precipitation profiles of Oenothera caespitosa subsp. montana, marginata and jonesii.

Figure 5. Precipitation profiles for Oenothera caespitosa subsp. caespitosa and purpurea.

Figure 6. Precipitation profiles of Oenothera caespitosa subsp. australis.

Figure 7. Precipitation profiles of Oenothera psammophila and Oe. eximia.

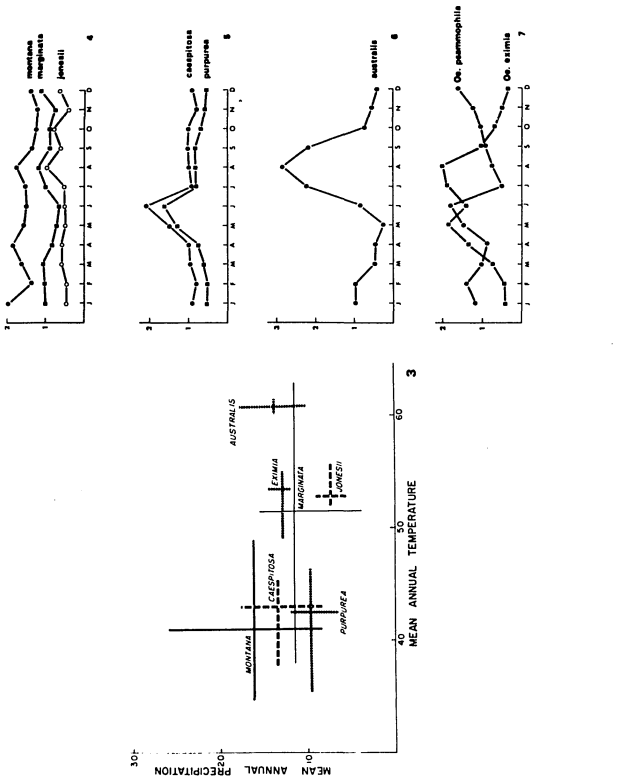


TABLE I. Average elevations and soil textures of Oenothera caespitosa, Oe. eximia and Oe. psammophila

Species	subspecies	Average elevation of known populations (ft)	Soil Texture (ave.) Mechanical Analysis %sand	%silt	%clay
caespitosa					
	australis	----	--	--	--
	caespitosa	6000	44	31	25
	crinita	9900	--	--	--
	jonesii	5100	63	21	16
	marginata	5600	57	28	15
	montana	6700	78	11	11
	purpurea	6000	27	50	23
eximia		6000	29	48	23
psammophila		6000	99+	--	--

Figure 8. Habitat of Oenothera caespitosa subsp. jonesii.

Figure 9. Habitat of Oenothera caespitosa subsp. purpurea.

Figure 10. Habitat of Oenothera caespitosa subsp. caespitosa.

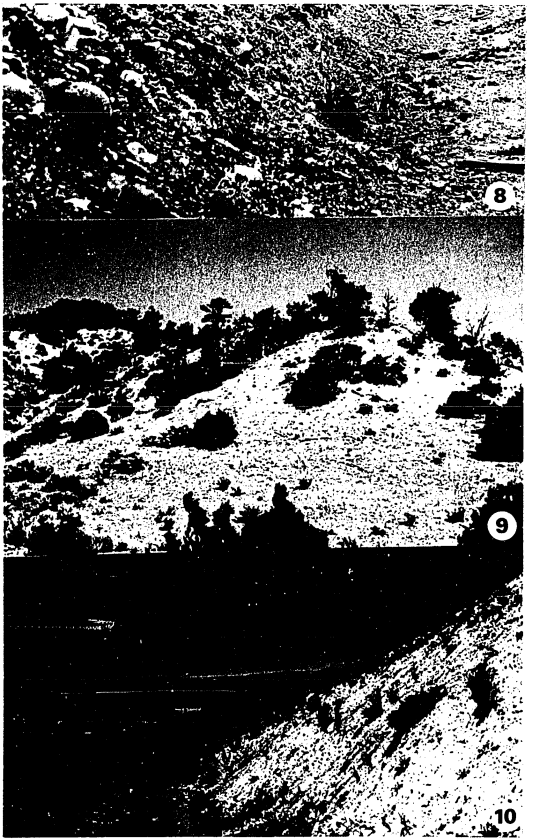
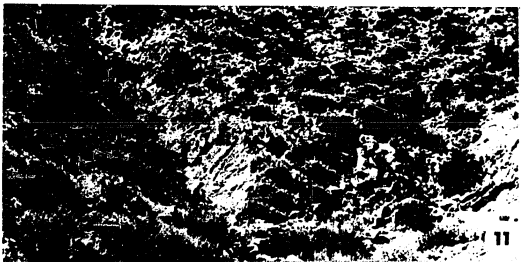
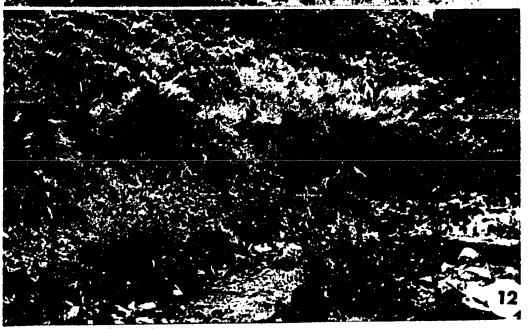


Figure 11. Habitat of Oenothera caespitosa subsp. marginata.

Figure 12. Habitat of Oenothera caespitosa subsp. montana.

Figure 13. Habitat of Oenothera psammophila.





sites on and among the dunes (Fig. 13). Psammophila is marginally sympatric with Oe. caespitosa subsp. caespitosa and marginata (Fig. 2). Populations of marginata were found to be growing within 200 yards of psammophila populations along the eastern edge of the dunes. The habitat of psammophila is characterized by high mean annual precipitation (14.6 in.) and low mean annual temperatures (42.0°F). Summers are hot and dry. The soil moistrue below a depth of about 18 inches generally drops below the wilting coefficient by late June (Chadwick and Dalke, 1965). Its precipitation profile is unique, with peaks in June and December (Fig. 7).

Oenothera eximia has a restricted range along the eastern edge of the southern Rockies from near Pikes Peak in Colorado, south into north central New Mexico. It is marginally sympatric with Oe. caespitosa subsp. montana in Colorado, and subsp. marginata along its southern boundary (Fig. 2). Soil requirements differ markedly with habitats of Oe. eximia having higher percentages of silt and clay and less sand than either montana or marginata (Table I). The mean annual temperature of eximia habitats (52.2°F) differs greatly from those of montana (40.8°F) but not from those of marginata (53.7°F). Habitats of eximia however, have a unique precipitation profile with a large peak, April through August, and dry winters, while those of montana and marginata have more linear profiles (Figs. 4 and 7).

Oenothera primiveris extends across the southern quarter of the western United States (Fig. 1). Primiveris is common at lower elevations (1,300-5,200 ft.) in southern California, Arizona, Nevada, Utah, New Mexico, Texas and northern Mexico. It is frequently found on open and disturbed sites along ephemeral streams and roadsides of the Mohave and Sonoran deserts. Its habitats are characterized by a mean annual precipitation of 7.7 inches (range 4.4 - 18.4) and a mean annual temperature of 65.9°F.

Oenothera cavernae has a very restricted distribution. It is edaphically isolated on calcareous flats and slopes, 1,500-4,000 feet, in Clark County, Nevada, and northwestern Arizona (Fig. 1) (Munz, 1965).

Oenothera xylocarpa is a narrow endemic found along the east flank of the southern Sierra Nevada in California and Nevada. It occurs on dry benches among the pines from 7,000 to 10,000 feet (Munz, 1965).

Oenothera brandegeei, Oe. macrosceles, Oe. muelleri, and Oe. tubifera are less known. Oenothera brandegeei has been reported from two sites in Mexico; Bahia de los Angeles on the east coast of Baja, California, and Isla Angel de la Guarda in the Gulf of California (Raven, 1970). In both cases it was found growing among volcanic rocks and stony ridges.

Oe. macrosceles and muelleri are sympatric along the central edges of their distributions in north central Mexico (Fig. 1). The two

are ecogeographically isolated. Macrosceles occurs in sites from 4,800 to 6,500 feet while muelleri occupies openings in the pine woods somewhat higher, 8,200-9,700 feet (Munz, 1965).

Oenothera tubifera has the most southern distribution of subgenus Pachylophus. It is found from 9,000 to 11,000 feet in Hildalgo and the Federal District of Mexico (Fig. 1)(Munz, 1931, 1965).

MORPHOLOGICAL STUDIES

Morphologically the members of subgenus Pachylophus are diverse. Duration of life, growth habit, leaf shape and pubescence, flower color and capsule morphology are characteristics which may be used to easily differentiate the various taxa.

Data from herbarium sheets, greenhouse propagations, personally collected materials and Munz (1931, 1965) has been summarized. These data clarify the taxonomic relationships among the taxa of subgenus Pachylophus.

The members of the Oe. caespitosa alliance, Oe. caespitosa, eximia, and psammophila, are morphologically distinct. Oenothera caespitosa possesses a great deal of heterogeneity. Morphological data for these taxa are summarized in Table II and growth habits and capsules are shown in Figs. 14-29.

Oe. eximia and Oe. psammophila are easily separated from the seven subspecies of Oe. caespitosa. Oe. eximia, in contrast to the

TABLE II. Morphological summary of subgenus Pachylophus.

Species	subspecies	Duration	Growth habit	diameter (cm)	Hypanthium length (cm)	Flower color fresh/after 24 hours	Capsules Tuberculate	Capsules Pedicellate	Leaf shape/ Pubescence	Compatibility
brandegeei		Annual	Acaulescent to caulescent	2-4	2-4.5	White/Pink	Yes	No	Oblanceolate deeply pinnatifid/ villous	Self-compatible Autog-amous
cavernae		Annual	Acaulescent	2-5	2-6	White/ Reddish	Yes	No	Oblanceolate/ pilose to glandular puberulent	Self-compatible Autog-amous
eximia		Annual	Caulescent and branched from base	4-9	3-10	White/ Pinkish	Yes	No	Oblanceolate/ pilose	Self-incompatible
primiveris		Annual	Caulescent and branching	2-8	3-6	Yellow/ orange-red	No	No	Oblanceolate/ pilose to subglabrous	Self-incompatible and Self-compatible
caespitosa										
	marginata	Perennial	Acaulescent to caulescent	6-12	4-16	White/Pink	Yes	Yes	Oblanceolate/ villous to hirsute	Self-incompatible
	jonesii	Perennial	Acaulescent	6-9	7-10	White/Pink to rose	Yes	Yes	Rhomboid ovate/ canescent to hirsute	Self-incompatible
	purpurea	Perennial	Acaulescent	5-9	5-7	White/Purple or deep rose	Yes	No	Oblanceolate/ canescent	Self-incompatible
	crinita	Perennial	Acaulescent	4-7	2-7	White/deep rose	Yes	No	Lanceolate/ hirsute to pilose	Self-incompatible
	montana	Perennial	Acaulescent	5-12	3-10	White/Pink	No	No	Oblanceolate/ villous on leaf margins	Self-incompatible
	caespitosa	Perennial	Acaulescent	5-8	3-8	White/Rose to deep rose	No	No	Oblanceolate/ Glabrous	Self-incompatible
	australis	Perennial	Acaulescent	8-14	10-20	White/--	---	No	Oblanceolate/ cinereous	Unknown
macrosceles		Perennial	Sub-caulescent with procumbent stems	6-9	9-13	Yellow/ Reddish	No	No	Oblanceolate/ glabrous with ciliate margins	Self-compatible modally outcrossing
mueller i		Perennial	Sub-caulescent with decumbent branches	9-13	12-20	White/Rose	No	No	Oblanceolate/ subglabrous	Self-incompatible and Self-compatible modally outcrossing
psammophila		Perennial	Caulescent and branched	4-8	4-7	White/Pink to rose	Yes	Varies	Oblanceolate/ glabrous	Self-incompatible
xylocarpa		Perennial	Acaulescent	2-5	5-7	Yellow/ Salmon red	No	No	Oblanceolate/ to obovate/ canescent	Self-incompatible
tubifera		Perennial	Sub-acaulescent to few branched	4-6	8-10	White/ Reddish	No	No	Linear to lanceolate/ strigose	Self-compatible

Figure 14. Growth habit of Oenothera psammophila (.5x).

Figure 15. Growth habit of Oenothera eximia (.3x).

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Figure 16. Growth habit of Oenothera caespitosa subsp. marginata (. 3x).

Figure 17. Growth habit of Oenothera caespitosa subsp. caespitosa (. 5x).

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Figures 18-25. Representative capsule drawings of 3 species of Oenothera.

Figure 18. Oenothera caespitosa subsp. marginata (1.3x).

Figure 19. Oenothera caespitosa subsp. caespitosa (1.4x).

Figure 20. Oenothera caespitosa subsp. montana (1.3x).

Figure 21. Oenothera caespitosa subsp. purpurea (1.5x).

Figure 22. Oenothera caespitosa subsp. crinita (1.5x).

Figure 23. Oentohera caespitosa subsp. jonesii (1.5x).

Figure 24. Oenothera psammophila (1.6x).

Figure 25. Oenothera eximia (1.2x).

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Figure 26. Growth habit of Oenothera caespitosa subsp. montana (.4x).

Figure 27. Growth habit of Oenothera caespitosa subsp. purpurea (.6x).

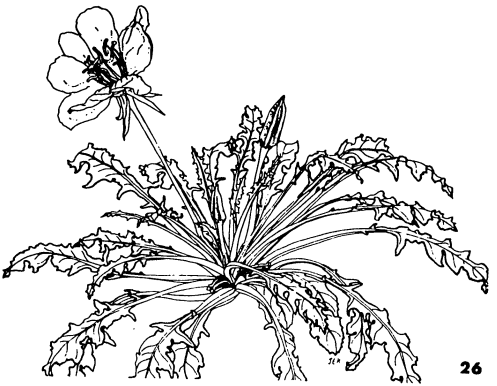

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Figure 28. Growth habit of Oenothera caespitosa subsp. crinita (.6x).

Figure 29. Growth habit of Oenothera caespitosa subsp. jonesii (.4x).

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other taxa is an annual. It is openly branched (Fig. 15) and 15 to 75 cm tall. The leaves are sinuate to pinnatified with pilose pubescence. Capsules are sessile with large tubercules (Fig. 25). The stem is also often red which is unusual in this alliance. In contrast to the other members of the alliance, eximia frequently has 10-20 flowers opening on the same night. Other taxa average 1-5 depending upon moisture and robustness of growth. Even its fragrance is distinct having been characterized as "gardenia like" by Goeken (1969).

Oe. psammophila is a caulescent, branching perennial (Fig. 14) with a well developed taproot. Its capsules are large, and sessile to pedicellate with low tubercules and many seeds (Fig. 24). In these characteristics its capsules are similar to those of Oe. caespitosa subsp. marginata (Fig. 18). Psammophila, however, is glabrous throughout with entire, lanceolate leaves. The apical leaves and buds are covered with a resinous exudate to which sand grains readily adhere. This forms a sand sheath which may serve to protect the apical meristem from the blowing sand. No other taxa within the alliance have a sand sheath. Petals are white, aging to deep pink or rose.

The seven subspecies of Oe. caespitosa form a complex in which many of the morphological characters tend to intergrade. Therefore, taxa are difficult to identify without flowers and mature fruit. Quantitative and qualitative differences have lead to many taxonomic difficulties among subspecies.

Oe. caespitosa subsp. marginata is easily separated from the other subspecies (Table II). Its growth habit ranges from caespitose to caulescent (Fig. 16). It often brances from the base. Plants of marginata are generally villous-hirsute throughout, especially on leaf margins, veins and hypanthia. Leaves are sinuate-pinnatifid. Capsules are large, many seeded, ridged with low to prominent tubercules and pedicelled (Fig. 18). Petals are white, aging pink.

The capsules of jonesii are slightly pedicelled and tuberculate resembling those of marginata. However, they are much smaller and are ovoid with a slightly flattened base (Fig. 23). Jonesii also differs from marginata in its growth habit (Fig. 29). Leaves are densely canescent to hirsute and rhomboid-ovate in shape. The rhomboid-ovate leaf shape is unique within the Oe. caespitosa complex.

Subspecies purpurea and crinita are both small, acaulescent perennials (Figs. 27 and 28) with sessile, tuberculate capsules (Figs. 21 and 22). The two are easily separated. Petals of purpurea age purple while those of crinita age rose to deep rose. Plants of purpurea are densely canescent throughout and have a purplish sheen which is unique among the subspecies. Crinita, on the other hand, has the smallest leaves (3-8 mm wide) of all the taxa. They are densely hirsute in pubescence.

Subspecies caespitosa and montana are the most difficult taxa to separate. Both are caespitose perennials (Figs. 17 and 26). Their

capsules are large, many seeded and sessile with low sinuate ridges, on their angles (Figs. 19 and 20). Their capsules are the only ones which are not tuberculed (Table II). Caespitosa and montana can be separated by leaf pubescence and margin shape; caespitosa is glabrous throughout and has sinuate leaves while montana has pubescent leaf margins and entire to sinuate-pinnatifid leaves. Petals of montana age pink and those of caespitosa age pink to deep rose.

Australis has the largest flowers of the complex (8-14 cm in diameter and hypanthia 10-20 cm in length). Its leaves are sinuate pinnatifid and finely cinereous.

Oe. primiveris, like Oe. caespitosa, is morphologically heterogeneous. Munz (1965) recognizes 3 subspecies, primiveris a small flowered (petals 10-22 mm long) caulescent form (Fig. 30); bufonis (E. M. Jones) Munz, a larger flowered (petals 25-40 mm long) acaulescent annual; and caulescens (Munz) Munz a large flowered (petals 30-40 mm long) caulescent taxon. These taxa seem to intergrade morphologically.

The remaining species of Pachylophus are relatively homogeneous. Oe. cavernae and Oe. brandegeei are both white flowered annuals (Table II). Morphologically they are similar but several differences are apparent. The leaves of brandegeei are distinctive in being deeply divided into narrow, acuminate, lobes which are directed forward, toward the apex of the leaf (Fig. 31). These lobes are much reduced,

Figure 30. Herbarium specimen of Oenothera primiveris subsp. bufonis (Type collection, M. E. Jones, (POM 38614)).

Figure 31. Growth habit of Oenothera brandegeei.

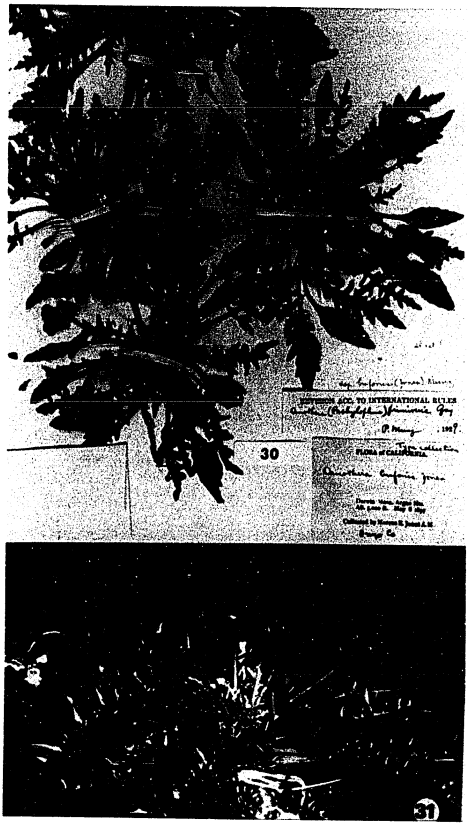

Figure 32. Herbarium specimen of Oenothera cavernae (Type collection, P. A. Munz (POM 255352)).

Figure 33. Herbarium specimen of Oenothera muelleri (Type collection, C. H. Mueller (POM 210702)).

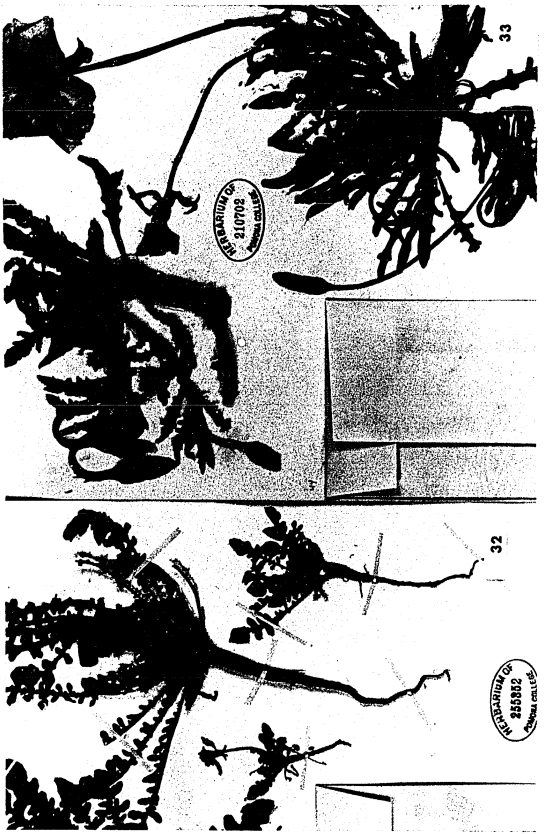

Figure 34. Herbarium specimen of Oenothera tubifera (J. N. Weaver, (POM 264876)).

Figure 35. Herbarium specimen of Oenothera xylocarpa (M. DeDecker (RSA 95916)).

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and the terminal lobe is very prominent in well-developed individuals. In Oe. cavernae, the lateral lobes are acute and obtuse and stand out at right angles to the rachis (Fig. 32). The terminal lobe of the leaf is much more prominent than in brandegeei. The capsules of the two taxa likewise differ modally, those of brandegeei being short and stout, 14-18 mm long, with very prominent, well separeted tubercules along the lines of dehiscence; those of Oe. cavernae are often longer, 15-38 mm long, with an acuminate apex and less prominent or distinct tubercules (Munz, 1965; Raven, 1970).

Oe. muelleri and tubifera are white flowered perennials with fleshy taproots and several decumbent or arching branches from a central rosette. (Fig. 33 and 34). The two species are nearly identical in both capsule morphology and habit. Flowers of muelleri however are much larger than those of tubifera (Table II). These two marginally sympatric taxa should be extensively investigated cytologically before further distinctions are made.

Oe. xylocarpa and macrosceles are yellow flowered perennials. They are easily separated morphologically. Oe. xylocarpa has a short caudex surmounted by leaves forming a crown at the ground-surface. Leaf blades are pinnately parted and often red spotted, with canescent pubescence (Fig. 35). The terminal lobe of the leaf is the largest. Flowers age salmon-red, a color unique within Pachylophus.

Oe. macrosceles has one to a few procumbent stems from a thick fleshy root (Fig. 36). Leaves are oblanceolate and glabrous except for ciliate margins. Petals age rose.

POLLINATION ECOLOGY STUDIES

Most of the members of the subgenus Pachylophus are outcrossing, self-incompatible (Table III) and hawkmoth pollinated. In general populations are small (10-100 individuals) and spatially isolated along mountain canyons, river courses and roadcuts.

The floral specializations which characterize the hawkmoth pollinated flowers of Oenothera are: crepuscular anthesis; large white flowers with exerted stamens; pollen connected by viscine threads; copious amounts of nectar secreted at the base of long hypanthia; self-incompatibility; and small numbers of flowers per population opening per night (Baker, 1961; Gregory, 1963-64; Faegri and van der Pijl, 1966).

Observations on the breeding systems and pollination ecology of Oe. caespitosa were initiated in 1971. Oe. caespitosa was found to be ideally suited to this study because it is composed of a polytypic group of outbreeding, colonizing subspecies, which are often found in isolated canyon populations in the Rocky Mountain region. Subspecies montana grows in close proximity to Ft. Collins, Colorado, and for this reason was the taxon chosen for intensive study. Observations were made at

Figure 36. Herbarium specimen of Oenothera macrosceles (R. McVaugh (RSA 141344)).

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PLANTS OF COAHUILA

Along rivulets at El Chorro, about 13 miles east of Saltillo, elev. ca. 1600 m.; shrubby. Flowers bright yellow.

TABLE III. Meiotic associations, pollen stainability and localities of natural populations used in this study.

Species	subspecies	Meiotic association	Pollen stainability	Locality and collector
			(ave. %)	
caespitosa				
	caespitosa	7II	87	Wyoming. Teton Co.: 1.1 mi. W of Grand Teton Nat'l Park on U. S. Hwy. 287, Stockhouse 133.
		7II	96	Wyoming. Fremont Co.: 15.1 mi. W of Dubois on U. S. Hwy. 287, Stock-house 134.
	crinita	7II	--	California. Inyo Co.: E. of Independence, Weins.
		7II	88	Klein 2654.
	marginata	7II	91	New Mexico. Santa Fe Co.: 10 mi. W of Santa Fe, Klein 1479.
		5II+04	90	Nevada. Nye Co., Hot Creek Range, 1 mi. NE of Marcy Peak, Klein 2612.
		5II+04	87	Nevada. Nye Co., Hot Creek Range, 2 mi. S of Marcy Peak, Klein 2642.

TABLE III. Continued.

Species	subspecies	Meiotic association	Pollen stainability (ave. %)	Locality and collector
		2n = 28	--	Colorado. El Paso Co.: 5 mi. up Rampart Range Road from Garden of the Gods, Stockhouse 115
		204+2ch+6II	75	Colorado. El Paso Co.: 8 mi. up Rampart Range Road from Garden of the Gods, Stockhouse 116.
		304+8II	83	Colorado. El Paso Co.: Gold Camp Road, Stockhouse 117.
		2n = 28	--	Colorado. El Paso Co.: Gold Camp Road, Bear Creek Canyon, Stockhouse 118.
		7II	--	Colorado, Boulder Co., 10 mi. W of Boulder on Hwy. 119, Stockhouse 123.
		7II	93	Colorado. Boulder Co.: 1.5 mi. E of Nederland, Stockhouse 121.
		7II	--	Colorado. Goeken 32.
		7II	93	Colorado. Trout Creek, Klein.
		2n = 28	87	Colorado. Green Mountain, Klein.

TABLE III. Continued.

Species subspecies	Meiotic associations	Pollen stainability	Locality and collector
		(ave. %)	
	2n = 14	93	Wyoming. Lincoln Co.: 7.5 mi. W of Opal on U. S. Hwy. 30N, Stockhouse 127.
	7II	--	Wyoming, Lincoln Co.: 7.2 mi. E of junction of U. S. Hwy. 89 & 30N, Stockhouse 128.
	7II	95	Utah. Grand Co.: 14 mi. E of Cisco, Stockhouse 214.
	7II	95	Idaho. Fremont Co., 5 mi. W of St. Anthony, Stockhouse 238.
	7II	95	Arizona. Coconino Co., in Page, Anderson.
	2n = 14	95	Idaho. Bannock Co., 1.8 mi. W of county line on U. S. 191, Stockhouse 553.
	7II	87	Rancho Santa Ana Botanic Garden, propagation number 9483.
	7II	90	New Mexico. McKinley Co., Bluewater Lake, Mollica 27.

TABLE III. Continued.

Species	subspecies	Meiotic association	Pollen stainability	Locality and collector
			(ave. %)	
	montana	7II	93	Colorado. Boulder Co., 1.4 mi. E of Nederland on U. S. Hwy. 119, Stock-house 121.
		7II	90	Colorado. Larimer Co., 3.1 mi. W of Bellvue, Stockhouse 105.
		7II	90	Colorado. Larimer Co.: Dixon Dam at Horsetooth Reservoir, Stockhouse 109.
		7II	92	Colorado. Larimer Co.: 3.5 mi. W of Bellvue, Stockhouse 114.
		7II	96	Colorado. Larimer Co.: 14.2 mi. S on road to Pingree Park from Colo. Hwy. 14, Stockhouse 119.
		2n = 28	84	Colorado. Teller Co.: 4 mi. W of Florisent, Stockhouse 110.
		7II	--	Colorado. Larimer Co.: 8.6 mi. S on road to Pingree Park from Colo. Hwy. 14, Stockhouse 120.

TABLE III. Continued.

Species	subspecies	Meiotic association	Pollen stainability (ave. %)	Locality and collector
		7II	--	Utah, 14 mi. W of Monticello, near Blue Creek, Stockhouse 537.
		--	71	Colorado. Jackson Co., 1 mi. NE of Rand, Stockhouse 527.
	jonesii	7II	92	Utah. Grand Co., Colorado River Canyon across from Salt Wash, Stockhouse 215.
		7II	90	Utah. Grand Co., Colorado River Canyon 1 mi. E of Salt Wash, Stockhouse 216.
		7II	90	Utah. Grand Co., Colorado River Canyon, 2 mi. W of Salt Wash, Stockhouse 532.
		7II	--	Utah. San Juan Co., Lake Powell, Stockhouse 549.
		14II, and 1I+7II+2IV+1V	25	Utah, San Juan Co., Blue Notch, Stockhouse 547.

TABLE III. Continued.

Species	subspecies	Meiotic association	Pollen stainability (ave. %)	Locality and collector
	purpurea	5II+ch4	91	Montana. Roadwater Co., 7 mi. E of Townsend on Hwy. 12. Stockhouse 131.
eximia		7II	97	Colorado. Fremont Co., 1 mi. E of junction Hwy. 120 & 115, Stockhouse 113.
		7II	--	Colorado. Fremont Co., 0.1 mi. E of junction of Hwy. 120 & 115, Stockhouse 112.
		7II	--	Colorado. El Paso Co., S entrance to Ft. Carson, Stockhouse 111.
		7II	92	Colorado. El Paso Co., 9.5 mi. S of Fountain on Hwy. 125, Stockhouse 137.
		7II	93	Colorado. Fremont Co., 1 mi. S of Florence, Goeken 18.
psammophila		7II	99	Idaho. Fremont Co., Dunes, 1 mi. N of Parker, Stockhouse 247.
		7II	95	Idaho. Fremont Co., Dunes, 1 mi. N of Parker, 2 mi. N of above site, Stockhouse 521a.

TABLE III. Continued.

Species subspecies	Meiotic association	Pollen stainability	Locality and collector
		(ave. %)	
	7II	95	Idaho. Fremont Co., 3 mi. W of 521a site, Stockhouse 521b.
	7II	97	Idaho. Fremont Co., 1 mi. SW of 521b site, Stockhouse 521c.
primiveris	5II+04	96	California, Eureka Valley dunes, Klein.
	4II+06	92	Arizona. Pima Co., 1 mi. S of Why, Stockhouse 290.
macrosceles	7II	95	Mexico. Stubbe.
muelleri	7II	94	Mexico. Stubbe.
tubifera	--	72	Mexico. Raven.
brandegeei	7II	89	Mexico. Angel de la Guarda, Gulf of California, Morgan 12983.

several populations in 1971. Additional populations from Utah and Idaho were added to the study in 1972. The breeding system of Oe. psammophila was also investigated.

Description of the study areas

Five localities of Oe. caespitosa subsp. montana were chosen west of Ft. Collins, Colorado, in 1971 for intensive study. Observations at these sites were continued through the summer of 1972.

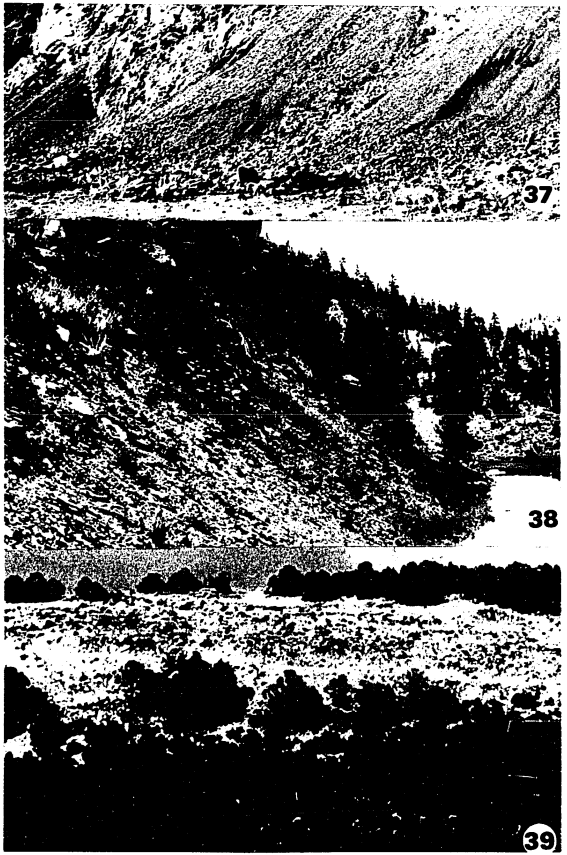
The first site was located approximately 3.5 miles west of the Colorado State University campus at Dixon Dam, Horsetooth Reservoir (5600 ft.). The vegetation of this area is predominantly mountain shrub dominated by Cercocarpus montanus. A small population of Oe. caespitosa subsp. montana grew along the disturbed interface between the dam and its supporting hogback. Approximately 75 plants of montana were scattered along a small runoff ditch down this interface. Several smaller populations are scattered along the base of the hogback for about 1/2 mile to the north.

A second intensive site was located 30 miles northwest of Ft. Collins, Colorado, along the Cache la Poudre River, in the montane zone dominated by Pinus ponderosa (elev. 6900 ft.). The site was an abandoned gravel quarry which has been excavated out of the canyon wall (Fig. 37). This population was small and was scattered on the open rocky slopes.

Figure 37. Abandoned gravel quarry 30 miles northwest of Ft. Collins, Colorado.

Figure 38. Population of Oenothera caespitosa subsp. montana from near Pingree Park, Colorado.

Figure 39. Disturbed hillside population of Oenothera caespitosa subsp. montana, 14 miles west of Monticello, Utah.



Three additional populations were located along Larimer County Road 131 which ends at Pingree Park. The populations were found on roadcuts through loose granite and are within the Ponderosa pine-Douglas fir community (8000-8500 ft.). Individuals of montana number between 75 and 100 at each site. A representative population from this area is shown in Figure 38. These populations were unusual in that Oe. coronopifolia and Oe. strigosa were often found in abundance with Oe. caespitosa subsp. montana.

Two colonies of Oe. caespitosa subsp. montana from Utah were studied intensively in 1972. They were located approximately 14 miles west of Monticello near Blue Creek, about 7 miles north of Mt. Linnaeus. The first population was small (50 individuals) and located 1/2 mile east of the second. The larger population (Fig. 39) was located in the oak brush belt dominated by Quercus gambelii. It was the largest population of Oe. caespitosa observed (greater than 700 individual plants). This population was approximately 100 yards long by 20-30 yards across and located in an area which had been cleared by the Forest Service in 1964.

Oenothera psammophila and Oe. caespitosa subsp. marginata were investigated from several populations at the sand dunes west of St. Anthony, Idaho. Plants of Oe. psammophila occur predominantly in blowout areas between the dunes and on sand hummocks (Fig. 40) dominated by Elymus flavescens. Populations of psammophila are large (200-500 individuals).

Figure 40. Sand hummoch inhabited by Oenothera psammophila, 7 miles west of St. Anthony, Idaho.

Figure 41. Lava outcrops in foreground inhabited by Oenothera caespitosa subsp. marginata.

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Along the eastern edge of the dunes, smaller populations of Oe. caespitosa subsp. marginata are found among the lava outcrops (Fig. 41). The two species often occur less than 200 yards apart.

Flower visitors and behavior

Hawkmoth visitors at the Oe. caespitosa populations differed from night to night and population to population. The percentage of total visits of the two hawkmoth genera to the caespitosa populations is summarized in Table IV. Sphinx vashti Strecker, S. asella (Rothchild & Jordan), S. chersis (Hubner) and Manduca quinquemaculata (Haworth) (in decreasing order of frequency) were the hawkmoths responsible for most of the pollination. Hyles lineata (Fabricius) and H. gallii (Rottenberg) were the only hawkmoths observed at populations of Oe. psammophila in Idaho.

The behavior of Sphinx and Manduca when visiting Oe. caespitosa subsp. montana was fairly consistent. The moths hovered, inserted their proboscis, and then, landed on the wide horizontal floral platform which was composed of the petals (Figs. 42 and 43). They often effect pollination as their proboscis, legs, or body brush the stigma. The three Sphinx species then thrust their head and body deep into the hypanthium in an effort to suck up as much nectar as possible. They frequently plunge so deep that only their wingtips protrude above the floral platform (Fig. 44). Manduca can withdraw most of the nectar without inserting its head into the floral tube because of its long

TABLE IV. Estimate of the percentage of total visits of moths to various populations of Oenothera caespitosa and Oe. psammophila.

Location	Moths and relative per cents per night			
	Sphinx	Manduca	Hyles	Noctuids
Colorado - Oe. caespitosa subsp. montana				
Horsetooth Reservoir	90-100	0-10	0	0
Poudre Canyon	80-100	0-20	0	0
Pingree Park	75-90	10-25	0	0
Utah - Oe. caespitosa subsp. montana				
Monticello	95-100	0-5	0	0
Idaho - Oe. caespitosa subsp. marginata				
St. Anthony dunes	90-100	0-10	0	0
Idaho - Oe. psammophila				
St. Anthony dunes	0	0	5-15	85-95

Figure 42. Sphinx sp. landing on Oenothera caespitosa subsp. montana.

Figure 43. Sphinx sp. taking off from Oenothera caespitosa subsp. montana.

Figure 44. Sphinx sp. plunging into hypanthial opening of Oenothera caespitosa subsp. montana.

Figure 45. Manduca quinquemaculata on floral platform of Oenothera caespitosa subsp. montana.

Figure 46. Evylaeus aberrans collecting pollen from Oenothera caespitosa subsp. montana.

Figure 47. Evylaeus aberrans with load of Oenothera caespitosa pollen.

proboscis (Fig. 45). Proboscides of Sphinx averaged 6 cm while those of Manduca were much longer, 11 cm.

The frequency of hawkmoth visits to montana flowers was observed to depend directly upon three environmental factors; wind, precipitation, and air temperature. Of the three, air temperature was the most important. Hawkmoth activity was greatest on warm nights. As the temperature dropped below 58°F hawkmoths became very scarce and at temperatures below approximately 54°F they were not observed. These data agree with laboratory experiments conducted with Manduca sexta by Heinrich (1971) who found that M. sexta was unable to generate sufficient thoracic temperature to fly at temperatures below approximately 54°F.

Hawkmoths were absent from all populations on nights when the wind velocity was high or gusting sporadically, as in advance of local thundershowers. Periods of light rain had little effect on hawkmoth activity on warm evenings. No hawkmoths were observed after heavy rainstorms.

Two species of crepuscular and matinal halictine bees Evylaeus aberrans (Crawford) and E. galpinseae (Cockerell), were collected at the Horsetooth locality. Both were observed primarily in the early mornings collecting pollen from subsp. montana (Figs. 46 and 47). E. aberrans was the only bee species collected at the

Pingree Park populations. No other bee species were observed visiting Oe. caespitosa throughout the course of this study.

Bohart (pers. comm.) found that E. aberrans is always associated with mountain environments and E. galpinseae with sandy flat environments such as occur near the shore line and along the roads at Horsetooth Reservoir.

On six successive evenings during the summer of 1971, an experiment was conducted at the Horsetooth populations to determine if these bees were pollinating subsp. montana. Fifty per cent of the flowers were bagged each night to exclued hawkmoths from them. The remaining flowers were allowed to open and were visited by hawkmoths. At 5:30 am the following mornings the bags were removed from those flowers which had been covered, and bees were seen shortly thereafter, actively collecting pollen. Capsules were marked on all flowers and were collected 1 1/2 months later.

It was found that only 1 out of 150 capsules set seed from the flowers which were visited only by bees. Approximately seventy per cent of the capsules developed from the flowers which were hawkmoth pollinated. Therefore, hawkmoths were found to be responsible for virtually all of the pollination which occurs in Oe. caespitosa subsp. montana. Bees (Evylaeus spp.) are pollen robbers and seem to play no significant role in the pollination of Oe. caespitosa. Gregory (1963-64) and Bohart (pers. comm.) have made similar conclusions from other species of Oenothera and bees.

Gene flow in natural populations

It has long been proposed that hawkmoths would be effective long distance vectors of pollen transfer in Oenothera and other genera (Rothchild and Jordon, 1903; Gregory, 1963-64; Ehrlich and Raven, 1969; Janzen, 1971; Heinrich and Raven, 1972). The extent to which the widely separated populations of the Oe. caespitosa alliance interbreed is of great importance in understanding evolution and speciation within this alliance.

Gregory (1963-64) suggested that individual hawkmoths wander over wide areas and are probably responsible for widespread cross-pollinations of Oenothera. He stated that "little direct evidence has been obtained to prove whether or not such transport takes place. . . ."

Field observations were made at populations of montana in Colorado and Utah to determine the rate of turnover of individuals in the local hawkmoth populations. Moths were captured on several successive nights, marked and released. Marked moths recaptured on successive nights were recorded (Table V).

There was rapid turnover in the hawkmoth populations at individual montana populations. More than 90 per cent of the hawkmoths entered and left the populations on a single night. Total numbers of hawkmoths captured on successive nights differed markedly from less than 10 to more than 40 each night (Table V). These results are similar to those of Gregory (1963-64), who investigated a population of

TABLE V. Summary of hawkmoth recapture studies.

Colorado		
Pingree Park populations (Oenothera caespitosa subsp. montana)		
7/14/1971 - 7/22/1971		
	46 Sphinx marked with yellow latex paint.	No moths captured had been previously marked.
Utah		
Monticello populations (Oenothera caespitosa subsp. montana)		
6/7/72	5 Sphinx captured and marked red.	
6/8/72	17 Sphinx captured, 15 marked red.	Two of the 17 moths collected had red marking from 6/7.
6/9/72	27 Sphinx captured and marked green.	None had any red marking from 6/7 or 6/8.
6/10/72	42 Sphinx captured.	3 had green marking from 6/9. 1 had red marking from either 6/7 or 6/8.
Idaho		
St. Anthony dune populations (Oenothera psammophila)		
6/14/72	5 Hyles lineata marked red	Five Hyles lineata were captured on 6/15 and 5 on 6/16 - none were marked with red.

Oe. hookeri and several different species of hawkmoths. A comparison of Gregory's observations with observations from this experiment suggests that turnover of hawkmoths in these populations is partially a function of the species of flower involved and the hawkmoth species, as well as the total flowers and amount of nectar present on a given night.

Intrapopulation pollination was examined at several populations of Oe. caespitosa subsp. montana in Colorado and Utah. Most of the observations were made at the large population of montana in Utah, near Monticello. The populations were divided into several sections and the flowers and pollen in each section were dusted with different colors of micronized dust before sunset. Hawkmoths were allowed to visit the populations during the night unmolested. The flowers were collected the following morning and then analyzed under ultraviolet light to detect the presence and quantity of the different colors of dust and dusted pollen. If, for example, a flower which was dusted red, had blue pollen on the stigma or blue dust on the flower, then it must have been visited by a hawkmoth which had first visited the blue flowers at the other end of a population. Both quantitative and qualitative measurements of pollen transfer (gene flow) within these populations were determined.

Hawkmoth visits were quantified by the following method. It was observed that one hawkmoth visit usually resulted in a small amount

of pollen or dust being deposited on the stigma. Two hawkmoth visits in an intermediate amount, and three visits on a large amount of pollen or dust being deposited. These qualitative data, light, medium, or heavy hits, were quantified to yield the number of hawkmoth visits to each flower studied.

The large populations of montana located west of Monticello, Utah, was divided into 5 sections to study intrapopulation gene flow. Flowers from one, two or three different sections were marked with different colors of micronized dust on given nights (Figs. 48-50).

On June 9, 1972, 26 flowers from the center (50 yd. mark) of the population were dusted with blue dust. Fifteen flowers were marked with red dust at the smaller population 1/2 mile to the east. The following morning flowers were collected and analyzed from both populations. Ten hawkmoth flights of 25 yards, and 12 flights of 50 yards each, to the north were recorded at the larger population. Twenty-five flights of 25 yards, and 10 flights of 50 yards were recorded to the south from the point of marking (Fig. 48). The wind on June 9, was observed to be predominantly from the west but fluctuated from the southwest. The number of hawkmoth flights in both directions within the population were nearly equivalent. The greater number of flights toward the south was probably due to the fluctuations in wind direction.

Eleven flights of 1/2 mile were recorded on June 9, in the upwind direction from the smaller population to the larger (Fig. 48). Three flights were detected in the downwind direction.

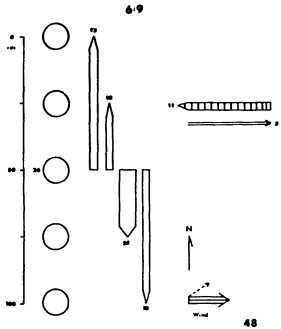
Figures 48-51. Summary of intrapopulation gene flow within Oe. caespitosa from near Monticello, Utah.

Figure 48. Intrapopulation gene flow, 6/9/72.

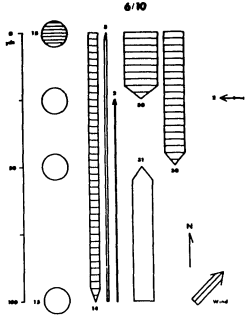
Figure 49. Intrapopulation gene flow, 6/10/72.

Figure 50. Intrapopulation gene flow, 6/11/72.

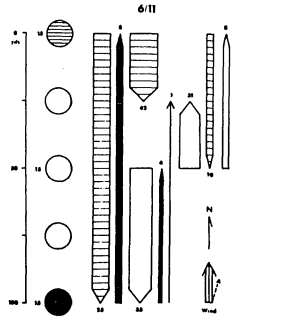
Figure 51. Summary of interpopulation gene flow in Oe. caespitosa from near Pingree Park, Colorado.



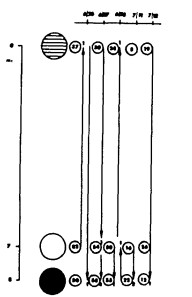
48



49



50



51

On June 10, 1972, flowers from both ends of the large population were marked (Fig. 49). Fifteen flowers were marked red in the vicinity of the zero yard line and 15 yellow at the other end of the population. Fifteen flowers were marked blue at the small population. The wind was from the southwest throughout the night.

The data shows that the majority of the hawkmoth flights were upwind (94 vs. 36 flights; Fig. 49). Two flights of 1/2 mile were recorded from the outlying population.

On June 11, 1972, three areas within the larger population were marked. The zero line was marked red (15 flowers), 15 flowers were marked blue near the 50 yard line, and 15 flowers yellow at the far end of the population. Fifteen flowers were marked green at the smaller population. The wind was from the south throughout the night (Fig. 50). Again, the majority of hawkmoth flights within the population were in the upwind direction (105 vs. 52; Fig. 50). No flights from, or to, the outlying population were recorded on this night.

The results from this study show that hawkmoths generally enter a population from the downwind direction and that gene flow was, for the most part in the upwind direction. Pollen dispersal was found to be non-random, those flowers which seemed to be more fragrant and/or more accessible were visited more frequently. Sphinx vashti, S. asella, S. cheris and Manduca quinquemaculata (in decreasing order of importance) were the hawkmoths which were responsible for most of the pollen transfer.

Interpopulation gene flow was first studied in 1971 at Horsetooth Reservoir and at the Pingree Park populations. Gene flow was approximated by the following experiment. Flowers were emasculated and hawkmoths were allowed to visit the populations on several nights unmolested. Capsules were marked, and then collected 1 1/2 months later, to determine seed set. Emasculation was divided into three classes on different nights. On successive nights 0%, 50% and 100% of the flowers in a population were emasculated. After 1 1/2 months the capsules were collected and the following results were observed. When none of the plants were emasculated 70% of the capsules set seed. When 50% of the plants were emasculated 40% of the capsules set seed, and when all of the flowers in a population were emasculated less than 7% of the capsules set seed.

The above data suggest that when all flowers are emasculated nearly 7% of the flowers are successfully fertilized by pollen from outside the population. It appears then that interpopulation gene flow is occurring. However, the source of the foreign pollen was unknown. It may have come from flowers which opened late and were therefore not emasculated, flowers which were only a short distance away, or from populations at some distance.

Experiments conducted during 1972 accurately defined the distance pollen was transported between populations. Experiments were conducted at Pingree Park with Oe. caespitosa subsp. montana and on the sand dunes west of St. Anthony, Idaho, with Oe. psammophila.

Interpopulation gene flow of 1/2 mile had already been documented during the experiments of interpopulation gene flow in Utah with subsp. montana. However, it was desirable to know to what extent widely separated populations were interbreeding. The three populations at Pingree Park were ideally suited for this type of study. The first and second population were separated by approximately 7 miles and the second and third by one mile (Fig. 51).

Flowers were marked at each population with different colors of micronized dust. Hawkmoths then visited the populations unmolested. Throughout these experiments the first population at the zero mile post was marked with red micronized dust, the second with blue and the third with yellow. For example, on June 20, 1972, 37 flowers were marked red, 82 blue, and 50 yellow (Fig. 51). One hawkmoth flight of seven miles was recorded from the second population to the first. During the course of these experiments three flights of seven miles were recorded, two flights of eight miles, and seven flights of one mile (Fig. 51). These results suggest widespread pollen transfer between populations.

Each night after the above populations were marked a black light insect trap was run in Poudre Canyon to collect any moths which might be in the area. On three successive nights, June 27-29 hawkmoths (Sphinx spp.) were captured which had yellow dust on their proboscides and bodies. These moths had flown at least 20 miles from the population

marked with yellow dust near Pingree Park. A total of 25 moths were captured on these three nights.

In summary, evidence of numerous flights was found between populations in both Colorado and Utah which were separated by distances of 1/2 and 1 mile. Several flights of 7 and 8 miles were recorded. Three hawkmoth flights of at least 20 miles were detected between Pingree Park and the Poudre River west of Ft. Collins, Colorado.

A similar marking experiment was conducted at populations of Oe. psammophila in Idaho. Populations along a trnasect through the dunes (southwest to northeast) separated by 1/4 mile, 1/2, 1, 2, 2 1/2, 3, 3 1/2 and 4 miles were studied. A total of 600 flowers were marked red at the first population, 300 marked blue at the 1 mile population and 400 marked yellow at the 4 mile population over a period of three nights. The results of this experiment were much different from those conducted with Oe. caespitosa subsp. montana.

The first difference was that no species of Sphinx or Manduca were observed. The only hawkmoths captured were Hyles lineata and H. gallii. These moths have shorter proboscides than those of Sphinx or Manduca and hover, rather than land on the corolla to get nectar. As a consequence of the hovering activity, less pollen and dust is transferred to the moth. The two moths were not very abundant. Only 15 moths were observed at the first population during the three evenings it was studied. Halictid bees and noctuid moths which were

very abundant, seem to be responsible for pollination of Oe. psammophila.

During the course of these experiments with Oe. psammophila intrapopulation gene flow was particularly apparent. Approximately 50-75% of the flowers in the three populations were marked. The different colors of dust were observed to be well distributed over their respective populations the following mornings. Those flowers which had not been marked the previous evening in these populations had been visited by moths which had visited the marked flowers. Noctuid moths and halictid bees seem to be responsible for most of the pollination which occurs. Hawkmoths (Hyles lineata and H. gallii) were not important pollinators.

Flowers from the three marked populations and the six unmarked populations were collected each morning. There were no flowers from the unmarked populations which had dust on them. It is concluded that interpopulation pollen dispersal was not occurring.

Hawkmoth floral constancy

The populations of Oe. caespitosa subsp. montana in Colorado near Pingree Park were ideally suited for the study of hawkmoth floral constancy. Oenothera caespitosa, Oe. coronopifolia T. & G. and Oe. strigosa (Rydb.) Mack & Bush, occurred in the same populations. Oe. coronopifolia is widespread caulescent roadside weed in Colorado. It is a perennial and is self-compatible but modally outcrossing. Flowers

open in a burst shortly after dark, one to two per plant per night. The flowers are pendant and structurally suited for bee pollination. Bees (Bombus sp.) and hummingbirds were observed visiting its flowers in the late evening and early morning. Noctuid moths were observed on its flowers at night. Bees and hummingbirds seem to be responsible for most of the pollination.

Oenothera strigosa is another widespread caulescent weed. It is a biennial and branches from the base. It is self-compatible and modally outcrossing. Flowers open in the early evening before those of caespitosa or coronopifolia and they are presented with the petals forming a vertical wall which is not conducive to visitation by the larger hawkmoths. The flowers of strigosa were often visited before sunset by hummingbirds, then later by hawkmoths (Hyles). Hummingbirds and bees (Bombus sp.) were frequent visitors in the morning. Evylaeus aberrans was also collected on strigosa.

Within these populations Epilobium and Cirsium were also abundant. Only bees were observed visiting Epilobium. Hummingbirds, bees and butterflies were frequent visitors to Cirsium.

Within these populations the larger hawkmoths, Manduca quinquemaculata, Sphinx asella, S. vashti and S. chersis were 100% constant to the Oe. caespitosa flowers. The horizontal landing platform presented by the petals seems to be important for these moths (Fig. 42), along with copious amounts of nectar. Oe. coronopifolia

was visited only by noctuid moths, bees (Bombus sp.) and hummingbirds.

The most important pollinators of strigosa seem to be hummingbirds and bees. Hawkmoths of the genus Hyles were observed regularly but the floral aspect of presentation does not seem to be conducive for pollination by these moths. Hyles was never observed visiting Oe. caespitosa, Manduca and Sphinx were never observed visiting strigosa or coronopifolia.

Hummingbirds played an important role in the pollination of strigosa and coronopifolia in these populations. They systematically foraged through the populations visiting flowers of one species consecutively then switching for awhile to the other nectar sources. They do not, however, visit Oe. caespitosa flowers. Again this is probably due to the aspect of floral presentation. The corolla of caespitosa forms a horizontal landing platform, while those of strigosa and coronopifolia are vertical. The hypanthium of caespitosa is much longer than in either strigosa or coronopifolia. Nectar is several centimeters from the top of the hypanthium in caespitosa and only moths with long proboscides can reach it.

Within these populations hawkmoths and hummingbirds were able to differentiate between contrasting floral forms and were constant to the different species of Oenothera. Hummingbirds and bees also differentiated between the different floral forms.

Ultraviolet reflectance

Horovitz and Cohen (1972) have shown the importance of ultraviolet reflecting patterns in the Cruciferae which act specifically as nectar guides for insects. The possible effect of variations in ultraviolet reflectance within a family or genus has only recently gained attention (Lindauer, 1967; Eisner, et al., 1969; Macior, 1971; Thompson, et al., 1972).

White and yellow are the most frequent petal colors in Oenothera. With varying amounts of ultraviolet reflectance they are likely to present a spectrum of patterns to the visiting insects.

Ultraviolet reflecting patterns from seven species of Oenothera were studied. Much diversity in intensity of the ultraviolet reflecting nectar guides was apparent within Pachylophus (Table VI). Oe. caespitosa, eximia, and brandegeei reflect weakly. Oe. primiveris on the other hand has very strong ultraviolet reflectance (Figs. 52 and 53). The differences in reflectance are probably associated with adaptations to different pollinators.

Oe. caespitosa and eximia are pollinated primarily by hawkmoths. The visual sensitivity of hawkmoths has not been intensively investigated (Meeuse, 1966). Oe. primiveris is visited by bees and hawkmoths (Gregory, 1963-64). It corolla is yellow, and other yellow flowered allogamous species of Oenothera also relfect strongly in the ultraviolet (Oe. maysillesei (Figs. 54 and 55) and Oe strigosa). Oe.

TABLE VI. Ultraviolet Reflectance of seven species of Oenothera.

Species	UV Reflectance	Breeding Behavior	Corolla color
brandegeei	+	Self-compatible autogamous	white
caespitosa subsp. marginata	+,++	Self-incompatible	white
caespitosa subsp. montana	+	Self-incompatible	white
eximia	+	Self-incompatible	white
maysillesii	+++	Self-compatible modally outcrossing	yellow
primiveris	++++	Self-comp. or incompatible modally outcrossing	yellow
strigosa	++++	Self-compatible modally outcrossing	yellow
triloba	+	Self-compatible autogamous	yellow

+ weak reflectance
++ medium reflectance
+++ strong reflectance
++++ very strong reflectance

Figures 52-57. Photographs of three taxa of Oenothera photographed in normal light (lefthand column) and ultra-violet light with an ultra-violet transmitting filter (righthand column).

Figure 52. Oenothera primiveris, normal light.

Figure 53. Oenothera primiveris, ultraviolet reflecting pattern.

Figure 54. Oenothera maysillesei, normal light

Figure 55. Oenothera maysillesei, ultraviolet reflecting pattern.

Figure 56. Oenothera brandegeei, normal light.

Figure 57. Oenothera brandegeei, ultraviolet reflecting pattern.

triloba (yellow flowered) and Oe. brandegeei (Figs. 56 and 57) have very weak reflectance which is to be expected of autogamous species (Table VI).

In general those species adapted to hawkmoth pollination reflect weakly in the ultraviolet. This may be because hawkmoths are not responsive to these wavelengths. Autogamous species also have weak reflecting patterns. However, this probably indicates their independence from insect pollinators. Those species which are visited extensively by bees reflect strongly in the ultraviolet. The bee eye does distinguish the ultraviolet as a color (Lutz, 1924, 1933; Lindauer, 1967; Eisner, et al., 1969; Horovitz and Cohen, 1972).

Nectar analysis

Little information is available on the sugar composition or nutritive value of nectars (Gilbert, 1972; Handel, et al., 1972). The nectar sugar composition of eight species of Oenothera are given in Table VII. The results indicate that qualitatively the eight species have identical nectar sugars. The nectars were composed of glucose, fructose, sucrose and an unknown. Quantitative measurements were not made, although it appeared from the chromatograms that there were differences in the quantity of the sugars produced among taxa.

The amount of nectar produced per night was determined for 4 species (Table VIII). Oe. caespitosa produced the largest volumes of nectar, averaging 35 microliters per flower (volumes were averaged

TABLE VII. Nectar composition of eight species of Oenothera.

Species	Glucose	Fructose	Sucrose	Unknown #1
albicaulis	+	+	+	+
caespitosa subsp.				
caespitosa	+	+	+	+
jonesii	+	+	+	+
marginata	+	+	+	+
purpurea	+	+	+	+
eximia	+	+	+	+
macrosceles	+	+	+	+
maysillesii	+	+	+	+
muelleri	+	+	+	+
primiveris	+	+	+	+
psammophila	+	+	+	+

TABLE VIII. Summary of the amount of nectar produced per night by several taxa of Pachylophus.

Species	%Sucrose	Nectar Volumes: Average in microliters	Nectar Volumes: Range in microliters	Average height of nectar in hypanthium (cm)	Average length of hypanthium (cm)
caespitosa	32.5	35	18-69	5.2	9.4
eximia	35.0	20	5-32	4.0	5.5
muelleri	29.5	20	17-23	3.0	16.0
primiveris	34.0	8	4-9	3.0	5.0

for subspecies jonesii, marginata and montana). Nectar was usually within 4 cm of the top of the hypanthium in Oe. caespitosa. Oe. eximia and muelleri averaged 20 microliters per night. Nectar of eximia was much nearer the hypanthial opening than in muelleri (Table VIII). Proboscid lengths for hawkmoths visiting Oe. muelleri must be very long (at least 13 cm) if they are to gain access to the nectar. Oe. primiveris produced on the average only 8 microliters of nectar per night.

The amount of nectar produced by the various taxa of Oenothera may be related to the different pollinators visiting them. Oe. caespitosa is pollinated by hawkmoths which require large amounts of energy (Heinrich and Raven, 1972). Oe. eximia and muelleri are also visited by hawkmoths. While individual flowers of eximia and muelleri produced much less nectar than Oe. caespitosa, they usually have 5-15 flowers open per plant per night in contrast to only 1-3 for Oe. caespitosa. Oe. primiveris is self-compatible and is less dependent upon insects, especially hawkmoths, in effecting pollination. Thus the lower yield of nectar is not unexpected.

Heinrich and Raven (1972) discussed the role of the energy budget of pollinators in relation to the food reward provided by the flowers they visit. Heinrich (1971) had determined that the 3 gram hawkmoth, Manduca sexta, expended approximately 11 calories of energy per minute while hovering and somewhat less while flying. Manduca

quinquemaculata and Sphinx chersis, both pollinators of Oe. caespitosa are approximately the same size as M. sexta and probably have similar energy requirements for flight.

The amount of energy available from a single flower of Oenothera caespitosa (ave.) was calculated. Flowers of Oe. caespitosa averaged 35 microliters of nectar per night, of which 32.5% was sugar (Table VII). Heinrich and Raven (1972) noted there are approximately 3.7 calories per mg of glucose. The following equation gives the number of calories per flower per plant each night available to hawkmoths: 35 mg nectar/flower x .325 (sugar concentration) x 3.7 calories/mg sugar = 42.1 calories per flower.

If the above estimate is even within 50% accuracy, the caloric reward of individual caespitosa flowers is fairly high. Even in small populations with only a few flowers open on a given night 42 calories should be sufficient energy reward for the hawkmoth pollinators.

An additional aspect of nectar composition and its nutritive value involves the extraction of amino acids from pollen. Gilbert (1972) found that butterflies of the genus Heliconius, through a simple feeding innovation, remove amino acids and proteins from pollen. This feeding innovation plays an important role in the reproductive and population biology of these insects. Free amino acids and protein were shown to be extracted with a nectar solution directly from the pollen.

It is believed that adult lepidopterans do not generally acquire amino acids and proteins from their food sources and therefore the nitrogenous compounds of their eggs are derived only from reserves laid down as a result of larval feeding (Davey, 1965; Engelman, 1970; Gilbert, 1972). When these reserves are exhausted, no further eggs are laid. Heliconius butterflies have solved this problem by extracting the essential amino acids directly from the pollen and in this way have extended their reproductive cycle by several months as compared to other butterflies (Gilbert, 1972). This resource is also important in extending the life span of Heliconius.

It became apparent after examining many hundred Oenothera caespitosa hypanthia in the field that hawkmoths visiting Oe. caespitosa may be deriving more than just nectar from the flowers. Very often hypanthia were found with large amounts of pollen within them. The pollen evidently falls into the hypanthium during pollination or during light breezes. Pollen adhering to the proboscis is also repeatedly dipped in and out of the nectar during the night. Pollen will release more than 50% of its free amino acids within minutes of being placed in a 10% sucrose solution (Standley and Linskens, 1965; Linskens and Schrauwen, 1969).

Nectar and pollen mixtures were examined for the presence of amino acids, especially proline. Nectar, nectar plus proline, nectar plus pollen and proline, and nectar plus pollen from Oe. caespitosa

were chromatographed (Fig. 58). The results of this experiment clearly show that the nectar of Oe. caespitosa does extract amino acids from the pollen. Proline was tentatively identified from this experiment (Fig. 58). This agrees with Kumar and Kecht (1967), who have shown that the pollen of Oenothera contains at least 8 free amino acids including large quantities of proline.

Nectar and pollen mixtures were chromatographed for three Oenothera species (Fig. 59). Serine, glycine, glutamic acid, proline and valine were tentatively identified. Several other amino acids remain unidentified.

The results indicate that hawkmoths may be obtaining a considerable diversity of amino acids from Oe. caespitosa and other species of Oenothera, in addition to the large caloric rewards necessary to keep such large insects in flight.

CYTOLOGICAL STUDIES

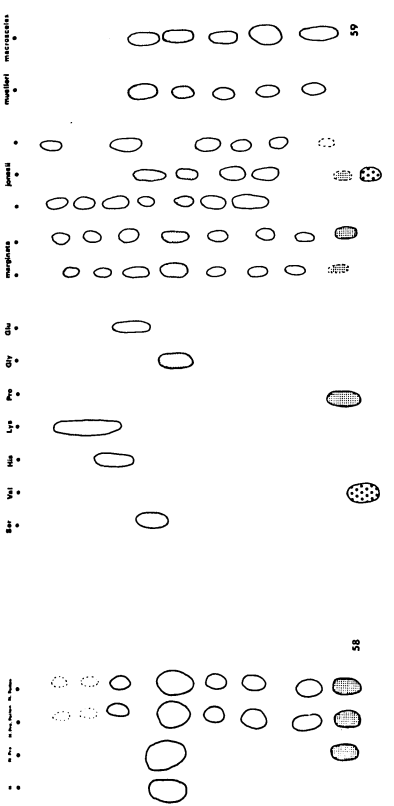
Parental types

Observations of meiotic chromosomes (diakinesis and metaphase I) were made on seven of the ten species of Pachylophus. A total of 53 populations were studied (Table III; Figs. 60-76).

Thirty-nine populations, widely distributed over the range of Oe. caespitosa were studied. Oe. caespitosa was among the species which were found to be structurally diverse. The majority of the populations

Figure 58. Chromatogram of nectar, nectar + proline, nectar + proline + pollen and nectar + pollen of Oenothera caespitosa.

Figure 59. Chromatogram of nectar + pollen of four taxa of Oenothera and standard amino acids.



Figures 60-76. Camera lucida drawings of meiotic configurations (x1000) of seven species of Pachylophus.

Figure 60. Oenothera brandegeei.

Figure 61. Oenothera psammophila (521a).

Figure 62. Oenothera primiveris subsp. bufonis (290).

Figure 63. Oenothera eximia (137).

Figure 64. Oenothera muelleri.

Figure 65. Oenothera macrosceles.

Figure 66. Oenothera caespitosa subsp. caespitosa (133).

Figure 67. Oenothera caespitosa subsp. crinita (2654).

Figure 68. Oenothera caespitosa subsp. jonesii (215).

Figure 69. Oenothera caespitosa subsp. jonesii (547).

Figure 70. Oenothera caespitosa subsp. montana (109).

Figure 71. Oenothera caespitosa subsp. montana (119).

Figure 72. Oenothera caespitosa subsp. montana (117).

Figure 73. Oenothera caespitosa subsp. montana (116).

Figure 74. Oenothera caespitosa subsp. marginata (238).

Figure 75. Oenothera caespitosa subsp. marginata (2612).

Figure 76. Oenothera caespitosa subsp. purpurea (131).

however, were found to be diploid (2n = 14) and structurally homozygous with seven pairs of chromosomes (7II). Structural heterozygotes (5II + 04 or a chain of 4), indicating a single reciprocal translocation, and tetraploids were found to characterize several populations (Table III).

Two of the 10 populations studied of subsp. marginata had individuals characterized by 5 pair and a ring or chain of 4 chromosomes indicating a single reciprocal translocation in each. The other 8 populations were found to be structurally homozygous (7II).

Subspecies montana, crinita and jonesii were found to have both diploid and tetraploid populations. Six populations of montana were shown to be tetraploid (maximum association, 304 + 1 ch4 + 6II). The tetraploid populations were found on dry sandy roadcuts primarily in the vicinity of Pikes Peak in Colorado. The ten additional populations of montana were diploid and structurally homozygous.

Plants from the two populations of subsp. crinita used in this study proved to be diploid and structurally homozygous (7II). Goeken (1969) reported a tetraploid population of crinita. Subsequent studies of this population by the present author, have revealed only the presence of diploid plants in that population.

Four populations of jonesii were found to be structurally homozygous (7II). The fifth was shown to be tetraploid with cells ranging from 14II to 1I + 7II + 2IV + 1V. This population was located in a

rather extreme environment on a precipitous slope leading from a monument rock near Lake Powell.

Two populations of subsp. caespitosa were studied and found to be structurally homozygous (7II).

The only plant of purpurea which was analyzed had a single reciprocal translocation (5II + ch4).

Living material of subsp. australis was not obtained for study.

Four populations each of Oe. eximia and Oe. psammophila were analyzed and found to be diploid and structurally homozygous (7II).

Single populations of Oe. brandegeei, Oe. macrosceles and Oe. macrosceles and Oe. muelleri were diploid and structurally homozygous (7II).

Two populations of Oe. primiveris were studied. Both were structurally heterozygous. The population of subsp. bufonis from Arizona had 4II + 06, indicating two reciprocal translocations. The other population from California, was characterized by 5II + 04 indicating a single reciprocal translocation.

Meiotic material of Oe. tubifera was not obtained. Living material of Oe. cavernae and Oe. xylocarpa was not studied. Seed of Oe. xylocarpa supplied by W. Stubbe failed to germinate.

Per cent stainable pollen was high for most material from natural populations (Table III). Tetraploid plants often had considerable reductions in per cent stainable pollen. They often had several pollen

grains (stainable and non-stainable) which were 4-lobed rather than the typical 3-lobed type in Oenothera. The highest percentage of 4-lobed pollen was found in 1 tetraploid population near Pikes Peak, in Colorado (17%).

Hybrid studies

Intra and interspecific crosses were made during the period 1969-1973. Fifty-one different intra and inter specific hybrid combinations were made. Over 1500 crosses were attempted, resulting in varying numbers of mature capsules and viable and non-viable seed (Tables IX and X). Meiotic chromosome configurations were determined along with pollen stainability for most of the hybrid combinations (Figs. 77-92, Tables XI and XII).

Intraspecific hybrids

Intraspecific hybrids between widely separated populations of Oe. eximia showed complete pairing and high pollen stainability (Table XI). These data indicate that chromosome restructuring in the form of reciprocal translocations has not occurred between populations of Oe. eximia.

Hybrids between the two widely separated populations of Oe. primiveris had a meiotic configuration of 2II + 04 + 06. Pollen stainability was reduced to only 10 percent in the hybrids (Table XI). These data indicate that the parental genomes differed by at least 3 reciprocal

TABLE IX. Summary of intra-subspecific crosses of Oenothera caespitosa.

Subspecies Crossed	Crosses producing plump seed: Crosses producing F_1	Crosses producing plump seed: Crosses from which seed was not germinated	Crosses producing plump seed: Crosses from which seed did not germinate	Crosses producing no seed	Total crosses attempted
caespitosa selfed	--	--	--	20	20
caespitosa x caespitosa	4	7	--	3	14
caespitosa x crinita	1	1	1	--	3
caespitosa x jonesii	4	3	--	2	9
caespitosa x marginata	10	10	5	12	37
caespitosa x montana	5	5	2	4	16
caespitosa x purpurea	3	6	3	2	14
crinita selfed	--	--	--	20	20
crinita x jonesii	--	--	--	2	2
crinita x marginata	3	3	3	3	12
crinita x montana	1	3	--	1	5
crinita x purpurea	--	--	1	--	1
jonesii selfed	--	--	--	27	27
jonesii x jonesii	--	5	--	--	5
jonesii x marginata	3	8	5	5	21
jonesii x montana	1	3	3	4	11
jonesii x purpurea	1	5	2	3	11
marginata selfed	--	--	--	105	105
marginata x marginata	6	30	3	10	49
marginata x montana	5	9	3	4	21
marginata x purpurea	6	18	3	8	35
montana selfed	--	--	--	116	116
montana x montana	8	8	2	4	22
montana x purpurea	5	6	1	2	14
purpurea selfed	--	--	--	7	7

TABLE X. Summary of inter-specific crosses within subgenus Pachylophus.

Subspecies Crossed	Crosses producing plump seed: Crosses producing F_1	Crosses producing plump seed: Crosses from which seed was not germinated	Crosses producing plump seed: Crosses from which seed did not germinate	Crosses producing no seed	Total crosses attempted
brandegeei selfed	7	40	3	5	55
brandegeei x caespitosa	5	10	6	27	48
brandegeei x eximia	--	1	4	5	10
brandegeei x primiveris	--	1	4	4	9
brandegeei x psammophila	--	--	--	5	5
caespitosa selfed	--	--	--	295	295
caespitosa x eximia	21	49	9	16	95
caespitosa x macrosceles	--	--	2	17	19
caespitosa x muelleri	--	--	4	9	13
caespitosa x primiveris	--	1	6	30	37
caespitosa x psammophila	8	9	1	40	58
eximia selfed	--	--	--	45	45
eximia x eximia	3	4	--	2	9
eximia x macrosceles	--	--	--	7	7
eximia x muelleri	--	--	--	5	5
eximia x primiveris	--	--	3	7	10
eximia x psammophila	5	2	--	10	17
macrosceles selfed	2	18	1	5	26
macrosceles x muelleri	3	2	1	6	12
macrosceles x primiveris	--	--	--	9	9
macrosceles x psammophila	--	--	--	5	5
muelleri selfed	--	--	--	20	20
muelleri x primiveris	--	--	--	6	6
muelleri x psammophila	--	--	--	4	4
primiveris selfed	5	35	3	16	59
primiveris x primiveris	4	5	--	5	14
psammophila selfed	--	--	--	25	25
tubifera selfed	--	5	--	---	5

TABLE XI. Chromosome pairing and stainable pollen of intraspecific hybrids.

Species	subspecies	Meiotic configuration	Percent stainable pollen: Number of plants examined	Range	Mean
caespitosa					
	caespitosa x caespitosa	7II	1	-	95
	caespitosa x jonesii	7II	5	73-97	89
	caespitosa x marginata	7II	5	50-87	80
	caespitosa x montana	7II	-	80-90	86
	caespitosa x purpurea	5II + 04	3	80-90	86
	crinita x marginata	7II	4	76-99	85
	crinita x montana	n = 7	1	66-88	74
	jonesii x purpurea	7II	2	57-83	70
	marginata x jonesii	7II	5	52-93	75
	marginata x marginata	7II	3	96-100	97
	marginata x montana	7II, 5II + 04	5	75-87	79
	marginata x purpurea	7II, 5II + 04	6	63-95	81
	montana x montana	7II	-	-	--
	montana x purpurea	7II, 5II + 04	2	75-96	85
eximia x eximia		7II	3	90-97	93
primiveris x primiveris		2II + 04 + 06	2	5-15	10

TABLE XII. Chromosome pairing and stainable pollen of interspecific hybrids.

Species	Meiotic configuration	Per cent stainable pollen: Number of plants examined	Range	Mean
brandegeei x caespitosa subsp. marginata	1II-30$\bar{4}$	5	0-2	1
eximia x caespitosa subsp. crinita	---	1	-	11
eximia x caespitosa subsp. marginata	7II, 5II-0$\bar{4}$	16	36-100	80
eximia x caespitosa subsp. montana	7II	7	63-95	80
eximia x caespitosa subsp. purpurea	---	3	50-92	72
eximia x psammophila	7II, 3II-20$\bar{4}$	6	40-93	63
psammophila x caespitosa subsp. jonesii	7II	2	60-93	83
psammophila x caespitosa subsp. marginata	7II	4	60-90	80

Figures 77-92. Meiotic configurations of F_1 hybrids (x1000).

Figure 77. Oenothera caespitosa subsp. caespitosa (133) x subsp. marginata (132).

Figure 78. Oenothera caespitosa subsp. caespitosa (133) x subsp. marginata (214).

Figure 79. Oenothera caespitosa subsp. montana (121) x subsp. caespitosa (133).

Figure 80. Oenothera caespitosa subsp. caespitosa (133) x subsp. purpurea (131).

Figure 81. Oenothera caespitosa subsp. crinita (2654) x subsp. marginata (214).

Figure 82. Oenothera caespitosa subsp. jonesii (215) x subsp. marginata (238).

Figure 83. Oenothera caespitosa subsp. montana (121) x subsp. marginata (132).

Figure 84. Oenothera caespitosa subsp. montana (109) x subsp. marginata (132).

Figure 85. Oenothera caespitosa subsp. marginata (127) x subsp. montana (Trout Creek).

Figure 86. Oenothera caespitosa subsp. montana (Trout Creek) x marginata (132).

Figure 87. Oenothera caespitosa subsp. marginata (132) x subsp. purpurea (131).

Figure 88. Oenothera caespitosa subsp. montana (Trout Creek) x subsp. purpurea (131).

Figure 89. Oenothera primiveris (290) x primiveris (Eureka Dunes).

Figure 90. Oenothera brandegeei x Oenothera caespitosa subsp. marginata (2612).

Figure 91. Oenothera caespitosa subsp. jonesii (215) x Oenothera psammophila.

Figure 92. Oenothera eximia (113) x Oenothera psammophila (521a).

translocations. There may also be genetic incompatibilities between the two genomes which have lead to the reduction in stainable pollen.

Intrasubspecific hybrids from widely separated populations of three of the seven subspecies of Oe. caespitosa were studied. The intrasubspecific hybrids of montana, marginata and caespitosa were found to be seven paired (structurally homozygous) and to have high pollen stainability (Table XI). These results suggest that subsp. caespitosa, marginata and montana are structurally homozygous throughout their ranges.

Hybrids from eleven different intersubspecific combinations of Oe. caespitosa were investigated (Tables IX and XI). These hybrids were for the most part structurally homozygous (7II). Pollen stainability was high even in those combinations resulting in ring formation. Morphologically the F_1 hybrids were found to be intermediate between their parents (Table XIII).

Several of the hybrids were characterized by rings of 4 chromosomes. Three of the four intersubspecific combinations involving subspecies purpurea resulted in progeny which segregated into two classes cytologically; those with 7II and those with 5II + 04. The single plant of subsp. purpurea used in this study was 5II + 04. Where sufficient hybrids were analyzed (purpurea x montana; purpurea x caespitosa; purpurea x marginata) segregation occurred. The only hybrid examined of purpurea x jonesii was seven paired. If more

TABLE XIII. Morphological summary of intrasubspecific hybrids.

Cross	Hybrid Morphology
caespitosa x crinita	Intermediate; leaves of crinita; pubescence variable.
caespitosa x jonesii	Leaves of jonesii; minutely pubescent to glabrous resembling caespitosa; perennial.
caespitosa x marginata	Leaves of caespitosa; minutely pubescent on leaf margins; capsules of caespitosa; caespitose to caulescent.
caespitosa x montana	Intermediate in pubescence; perennial.
caespitosa x purpurea	Intermediate; leaves and pubescence variable but often resembling purpurea.
crinita x marginata	Intermediate; robust; leaves and pubescence in general intermediate.
crinita x montana	Intermediate; leaves of montana; pubescence of crinita.
jonesii x purpurea	Leaves intermediate; pubescence of purpurea; pubescent to glabrous buds.
marginata x jonesii	Leaves strongly resemble jonesii in shape and pubescence; more robust in appearance than jonesii.
marginata x montana	Intermediate; leaves and pubescence variable; capsules of marginata often caulescent.
marginata x purpurea	Leaves intermediate and variable in shape; pubescence of purpurea; buds and leaf tips red; buds variable, glabrous to villous; capsules with red dots.
montana x purpurea	Intermediate; leaves of montana; leaf pubescence glabrous to minutely pubescent on margins; capsule of montana.

material were available, segregation would probably have been observed.

The only other intersubspecific cross resulting in a ring formation involved subspecies montana and marginata. One of six different combinations resulted in ring formation (04). This combination was between the Trout Creek population of subsp. montana and the Opal (127) population of subsp. marginata. Meiotic chromosome data for the Opal population was not obtained (Table III). The data indicates these two populations differ by a single reciprocal translocation. It is likely then, that the Opal population of subsp. marginata has a ring of 4 chromosomes or that its chromosome end arrangement differs from that of the Trout Creek population of subsp. montana. All other combinations between different populations of montana and marginata were seven paired.

These data indicate that the subspecies of Oenothera caespitosa share a common chromosomal end arrangement throughout their ranges with only the occasional occurrence of structurally heterozygous populations.

Interspecific hybrids

Interspecific crosses involving seven of the taxa of Pachylophus were investigated (Tables X and XII). A morphologic summary of F_1 characteristics is presented in Table XIV. The interspecific hybrids

TABLE XIV. Morphological summary of interspecific hybrids.

Cross	Hybrid Morphology
brandegeei x caespitosa	In general the appearance of the hybrids strongly resembled brandegeei; leaves intermediate but pinnatifid like those of brandegeii; pubescence on margins like caespitosa; buds and leaves with red dots like brandegeei; subcaulescent and branching; self-compatible.
brandegeei x eximia	Weak; died after two weeks.
caespitosa x eximia	Intermediate; leaf shape and vesture variable, primarily that of eximia; stems red like eximia; capsules of eximia; often weak with pale yellow-green color; caulescent with occasional branching.
caespitosa x psammophila	Intermediate; leaf shape primarily that of psammophila; pubescence of caespitosa; buds often minutely pubescent or glabrous; leaves without the resinous exudate of psammophila.
eximia x psammophila	Intermediate; leaf shape and vesture variable; buds glabrous; leaves without the resinous exudate of psammophila; caulescent with well developed lateral branches.
macrosceles x muelleri	Seedlings tricotyledonous and white; died after one week.

were in general found to be intermediate in morphology between their parents.

Interspecific crosses were attempted between Oe. brandegeei and Oe. caespitosa, eximia, primiveris and psammophila. Oe. brandegeei x caespitosa was the only cross resulting in viable seed (Table X). Cytologically these hybrids were found to have 1II + 304, and a great reduction in stainable pollen, 0-2% (Table XII). These data indicate that brandegeei and caespitosa differ by three reciprocal translocations and may also be genetically incompatible. Stubbe (pers. comm.) has been unable to cross Oe. brandegeei and caespitosa.

Oenothera eximia was successfully crossed to four different subspecies of Oenothera caespitosa (Table XII). Most of the hybrids between Oe. eximia and Oe. caespitosa subsp. marginata were found to be seven paired. A single cross, Oe. eximia (113) x Oe. caespitosa subsp. marginata (Bluewater Lake) resulted in a hybrid with a ring of four indicating a single reciprocal translocation difference between the two populations. The evidence indicates that the Bluewater Lake population of marginata has probably undergone limited chromosome restructuring since the other crosses involving Oe. eximia and subsp. marginata were seven paired.

Hybrids between Oe. eximia and subsp. montana were structurally homozygous (7II) with high pollen stainability (Table XII). Meiotic data for eximia x subsp. crinita and subsp. purpurea were not obtained.

Hybrids from the eximia x crinita cross showed a large reduction in stainable pollen. This may indicate a shift in chromosome end arrangement for crinita, or genetical incompatibilities between the two taxa.

Interspecific crosses between Oe. eximia and macrosceles, muelleri and primiveris resulted in either no seed being produced or in the production of inviable seed (Table X).

Hybrids between Oe. eximia and Oe. psammophila were found to be variable in meiotic configuration and pollen stainability (Table XII). Two hybrids (113 x 247) were seven paired with high pollen stainability (70-93%). Four hybrids (137 x 521a) had meiotic configurations of 3II + 2I and low pollen stainability (40-50%) indicating a difference of two reciprocal translocations between the populations.

Intraspecific crosses of Oe. eximia (113 x 137) have shown that eximia is structurally homozygous. Intraspecific data is not available for Oe. psammophila. These data involving different populations of eximia and psammophila suggest that it is Oe. psammophila which has undergone structural rearrangement. It is possible that different populations of psammophila have different end arrangements, even though they are seven paired.

Those hybrids which had the two rings of four chromosomes were weak with yellowish-green leaves. Most crosses between psammophila and eximia resulted in shriveled seed. This possibly indicates the presence of genetical incompatibilities between the two taxa, which

have been established by the chromosomal rearrangement of this population of Oe. psammophila.

Oenothera psammophila was successfully crossed with only two taxa of Oe. caespitosa (Table XII). Hybrids of Oe. psammophila (247) x Oe. caespitosa subsp. jonesii and subsp. marginata were structurally homozygous with high pollen stainability. Numerous crosses with the other subspecies of Oe. caespitosa were attempted but none resulted in seed set. In most of the crosses involving psammophila, the seed which was obtained was shriveled and inviable. Few viable seeds were obtained even in those crosses which were compatible. The difficulty in acquiring viable F_1 seed from interspecific crosses with psammophila probably indicates cytological or genetical incompatibilities between it and the other taxa.

All seed derived from interspecific crosses between Oe. caespitosa and macrosceles, muelleri and primiveris was inviable (Table X).

Oenothera macrosceles was successfully crossed with Oe. muelleri but the hybrids were white and died within a few days. They had three cotyledons. These results suggest plastid-cytoplasmic incompatibilities and/or genetical and cytological anomolies between the two genomes. Stubbe (pers. comm.) has had similar results with crosses involving macrosceles and muelleri. In the present study

macrosceles could not be crossed with primiveris or psammophila (Table X).

All interspecific crosses between muelleri and primiveris, and muelleri and psammophila were unsuccessful (Table X).

Differentiation within the polytypic complex of Oe. caespitosa has occurred within the framework of a single chromosomal arrangement which appears as the structurally homozygous configuration in most populations. Intersubspecific hybrids were highly fertile. Differentiation within the polytypic complex of Oe. primiveris is associated with heterozygous restructuring between populations.

Interspecific hybridization indicated that species differ from none, to at least 3 reciprocal translocations. Those which were interfertile showed a more marked reduction in the per cent stainable pollen than was observed in the intersubspecific combinations.

EVOLUTIONARY TRENDS IN PACHYLOPHUS AND PHYLOGENETIC IMPLICATIONS

REVIEW

Data from studies of morphology, ecology, cytology breeding and pollination ecology have provided evidence which clarifies the taxonomic and phylogenetic relationships within the subgenus Pachylophus. The species were in general found to be marginally sympatric over wide geographic areas, however, they are ecologically isolated from each other by their varying habitat requirements. In addition, limited genetic restructuring, and certain pollinator preferences have acted as barriers restricting gene exchange between species.

Morphologically, both Oenothera caespitosa and Oe. primiveris were characterized as heterogeneous and polymorphic. Morphological characteristics within each species tend to intergrade among subspecies. However, taxa can be separated from one another by modal differences in these characteristics. The remaining species of Pachylophus were each morphologically homogeneous.

Ecologically, the members of Pachylophus seem to be isolated from one another by habitat differences which may help restrict gene flow between them, especially among the subspecies of Oe. caespitosa. Ecological isolation has probably been one of the most important factors

influencing evolution within Pachylophus, especially within Oe. caespitosa. Stebbins (1950) and Grant (1963, 1971) have emphasized the importance of ecological differentiation in establishing isolating barriers between populations which are not genetically isolated. They emphasize that ecological differentiation as a barrier to gene exchange can set the stage for the formation of other isolating mechanisms.

Cytologically, the species of Pachylophus are generally characterized by the structurally homozygous condition (7II). Only occasional structural heterozygotes were found. Oenothera caespitosa and Oe. eximia were found to be structurally homozygous and to share a common chromosomal end arrangement. The general trend of hybrid sterility and non-crossability of the remaining taxa probably indicates cytogenetic incompatibilities or chromosomal restructuring among these taxa. When compared to the more advanced subgenera of Oenothera, chromosomal restructuring has been minimal in Pachylophus.

Studies of the pollination ecology of several of the taxa have shown that certain pollinator preferences are manifested among taxa. Associated with the differentiation of the breeding systems of the taxa of Pachylophus are changes in compatibility from the allogamous, self incompatible species (Oe. caespitosa, Oe. eximia, Oe. psammophila), to those which are self compatible and autogamous (Oe. cavernae and Oe. brandegeei).

Although the evidence indicates a close relationship among the taxa of the subgenus Pachylophus, especially Oe. caespitosa with eximia, psammophila, brandegeei and cavernae, discontinuities between taxa have led to a rather complex phylogeny.

EVOLUTIONARY TRENDS

Evolution within the subgenus Pachylophus has been in the form of several general trends. Morphologically, there have been changes in life cycle (perennial to annual), changes from large, many seeded capsules to smaller, fewer seeded ones, from large flowers to smaller ones, and changes in pubescence. Ecologically, there have been shifts from intermediate habitats such as those occupied by Oe. caespitosa subsp. marginata to more xeric (Oe. psammophila) and more mesic habitats (Oe. xylocarpa and Oe. caespitosa subsp. montana). There have also been edaphic shifts (Oe. cavernae, Oe. eximia, Oe. psammophila and Oe. xylocarpa).

PRIMITIVE ANCESTRY AND PHYLOGENY

Zinmeister and Bartl (1971) and Howard and Mabry (1972) have shown the subgenus Pachylophus has myricetin. This highly oxygenated flavonoid is not found in the more advanced subgenera of Oenothera. The loss of myricetin is believed to be an advancement in the evolution of flavonoids in the higher plants in general (Zinmeister and Bartl, 1971).

Therefore, its presence in Pachylophus may indicate that this subgenus is primitive.

Klein (pers. comm.) suggested that morphologically the subgenus Pachylophus is possibly one of the most primitive subgenera of Oenothera. Cytological evidence from the present study also supports the primitive position of the subgenus Pachylophus.

Cytologically the species of Pachylophus, in general, are characterized by structurally homozygous chromosomes which form seven bivalents at meiosis. The more advanced subgenera, such as Oenothera and Raimannia, are characterized by large rings at meiosis and structurally heterozygous chromosomes. Darlington (1929) and Clelend (1957, 1972) have concluded that cytologically the primitive mode in Oenothera was structural homozygosity with either no rings or small rings at meiosis.

Thus, biochemical, morphological and cytological evidence indicates that Pachylophus is a primitive subgenus.

Cleland (1957, 1972) and Raven (1962) have concluded that the primitive condition within the genus Oenothera can be characterized by a taxon which is outcrossing, self incompatible and chromosomally structurally homozygous. They also conclude that this taxon would most probably be a perennial, with large flowers and large many seeded capsules. It can be inferred from Lewis and Raven (1958) and Klein (1970) that the more primitive taxa in Oenothera would also be

ecologically, morphologically and cytologically more diverse than the other taxa.

Within the subgenus Pachylophus, Oenothera caespitosa most closely approximates the above description of a primitive taxon. Ecologically, cytologically and morphologically Oe. caespitosa was found to be the most diverse taxon within Pachylophus. It was found to occur in habitats with the widest range of mean annual temperatures and precipitation, and it has the widest geographic range. Subspecies marginata is probably the most primitive of the seven taxa of Oe. caespitosa. It has been characterized as occupying habitats which are in general intermediate to those of the other subspecies of caespitosa.

Stebbins (1959) concluded that ancestral taxa, in general, are often found in or are believed to have occupied intermediate habitats as compared to habitats of more advanced taxa. Thus, marginata is probably the most primitive extant taxa in Pachylophus.

The southwestern United States is presently the area of greatest species diversity for the subgenus Pachylophus. For this reason Pachylophus probably had its origin in this area. The ancestral home of the genus as a whole has been recognized to be in Mexico or the far southwestern United States (Geckler, 1950; Cleland and Hammond, 1950; Cleland, 1957, 1972; Raven, 1962). The origin of Pachylophus is consistent with the origin of the genus.

Raven (1962) has suggested that the older elements of Oenothera differentiated or made their initial appearances with the Madro-Tertiary geoflora in the southern Rocky Mountains and adjacent Mexico. He concludes that the evolution of the primitive Oenotheras probably proceded with the differentiation of the Madro-Tertiary geoflora. Lewis (1953a, 1953b) suggested a corresponding time for the differentiation of the closely related genus Clarkia which occurs in habitats like those of many species of Oenothera.

The primitive elements of the Oenothera caespitosa alliance may have been associated with certain aspects of arid subtropical scrub in the southwest. According to Axelrod (1957, 1958) this vegetation type was common well into Pliocene time. The pinon-juniper woodland which had its origin earlier became fully differentiated in the late Pleistocene (Axelrod, 1958). Subspecies marginata presently occupies this vegetation type and may have been associated with it since its origin.

Ecologically disturbed sites were abundant throughout the southwest during the Pliocene and Pleistocene (Axelrod, 1957, 1958). These areas were much more mesic then than at present (Antevs, 1954; Gray, 1961). It is probable that Oenothera caespitosa or the primitive caespitosa elements were adapted to these disturbed sites under this climatic regime.

The remaining taxa of Pachylophus probably differentiated from this ancestral Oenothera caespitosa complex. This may have occurred as the Pacific storm tract migrated northward and the climate and vegetation during the Pleistocene underwent catastrophic fluctuations (Antevs, 1954; Darrow, 1961; Gray, 1961; Martin, 1961; King, 1964; Morrison, 1965). It was also during this period that the deserts of the southwest probably reached their present extent.

SELECTION PRESSURES IN A CHANGING ENVIRONMENT

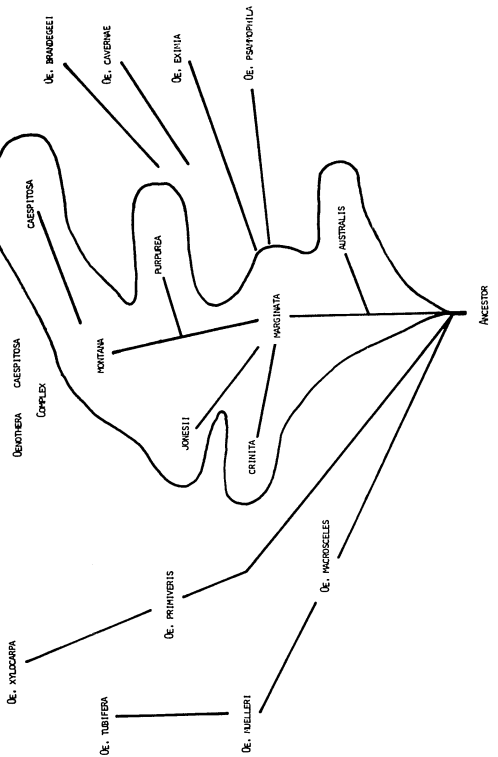
Differentiation of Oenothera caespitosa, the most widespread and polytypic species of Pachylophus, was probably promoted by the late Pleistocene trend toward increasing aridity in the southwest. The various subspecies of the Oe. caespitosa complex most likely differentiated during the postpluvial periods in response to selective pressures associated with the rapidly changing environment of this area. Rapid and catastrophic environmental fluctuations probably enhanced selection between different edaphic modes and different temperature and precipitation regimes. Initially, spatial isolation was strengthened as ecogeographic isolation became more apparent and gene flow between populations decreased. Certain pollinator preferences, such as exhibited by hawkmoths of the genera Manduca, Sphinx and Hyles, in mixed populations of Oenothera, may have further enhanced this isolation. Ethological isolation may have led to some of the ecological and morphological differences between taxa.

Pollinator preferences may have strengthened initial ecogeographic or edaphic discontinuities between the taxa of Pachylophus. The possibility of sympatric differentiation in other plant groups resulting from preferential activities of pollinators has been suggested by several authors (Grant, 1949; Straw, 1955; Raven, 1962; Ehrlich and Raven, 1969). Grant (1950) has pointed out that more rapid species formation might be expected among plants associated with floral constant visitors than among those pollinated by inconstant ones. Ethological isolation probably never provides the primary barrier which initiates speciation, but rather acts in reinforcement of some other barrier (Grant, 1949). Initial ecogeographic isolation, followed by the development of ethological isolation in Pachylophus has undoubtedly strengthened discontinuities between several of the taxa.

PHYLOGENETIC RELATIONSHIPS WITHIN OENOTHERA CAESPITOSA

Morphological, ecological and breeding data from this study suggest that the taxa of the Oenothera caespitosa complex were derived from marginata or a marginata-like ancestor. Subspecies marginata has been characterized as closest to the ancestral condition and for this reason probably differentiated early. Subspecies australis, crinita, jonesii and montana are likely of early origin (Fig. 93).

Figure 93. Phylogenetic relationships of the ten species of subgenus Pachylophus.



The time period during which the pinon-juniper woodland was undergoing extensive migrations seems a likely point for the differentiation of both australis and montana. As the Pacific storm tract moved north during the late Pleistocene, southeastern Arizona and New Mexico became progressively more xeric (Gray, 1961). It is likely that subspecies australis became stranded in the wetter mountains of this area as these changes in the environment occurred. Morphologically, its caespitose, perennial growth habit, large flowers and dense pubescence, suggest that it is indeed an older member of the caespitosa complex.

Subspecies montana occupies the most mesic habitats of all the subspecies. It is found in areas of high rainfall including a winter precipitation component (Fig. 4). The fluctuations in vegetation associated with mountain glaciation in the Rockies and the rapid development of disturbed habitats (Ray, 1940; Antevs, 1954; Richmond, 1960) probably created ideal conditions for the spread of montana.

In view of the many more or less disjunct populations of montana, crinita and jonesii across the southwestern desert mountains, Great Basin and Colorado Plateau, these taxa were probably at one time more widespread then at present. Most likely this last occurred during the Wisconsin glacial when this area was more mesic. Since that time their ranges have contracted.

A similar situation is found in Clarkia (Mosquin, 1964). Clarkia rhomboidea is presently found in California, Washington and Idaho

along the edges of the Great Basin and isolated in widely separated populations in Idaho, Utah and Arizona. Mosquin believes that during the Wisconsin glacial, these populations were contiguous throughout the area. As the "wane of the effects of glaciation brought increasing aridity to the climate of the western United States", C. rhomboidea invaded those habitats which remained more mesic.

It is probable that this same sequence of events has led to the isolated populations of Oe. caespitosa in and around the Great Basin area.

Cytological evidence also points to an early origin for montana. There are many polyploid populations in the vicinity of Pikes Peak, Colorado. Morphologically, these are identical to the diploid populations. In this same area, diploid populations are found in the moist canyons while the polyploid populations occupy more xeric habitats on exposed gravely road cuts. The situation is the same for subsp. jonesii.

Similar results are reported by Mosquin (1966). He found that the diploid populations of Epilobium angustifolium (Onagraceae) occupy the colder and more mesic habitats while autotetraploid populations occupy the more xeric habitats.

Laws (1965, 1967) has shown that with induced tetraploids of Oenothera affinis, there is an increase in the number of germinal pores in the pollen. She suggests that as autopolyploid population ages, selection is high for the triaperaturate condition rather than for the greater

numbers of pores. The pollen from plants of several of the tetraploid montana populations in the present study were found to be triaperaturate (80-100%) suggesting a long period of isolation since their differentiation from diploid stock.

The tetraploids of crinita, jonesii and montana are most likely of autoploid origin. Most experimentally produced autopolyploids (Hecht, 1944; Mosquin, 1966; Small, 1966, 1967) are almost indistinguishable morphologically from the diploid specimens. Inter-racial hybridization often releases considerable morphological variability in tetraploids (Mosquin, 1966). The tetraploids of subspecies montana and jonesii were not found to be any more variable than the diploids of these taxa. Cytologically the quadrivalent associations of montana and jonesii (Figs. 69, 72 and 73) also point to autopolyploid origin.

Stebbins (1957) in discussing the occurrence of autopolyploidy in the flowering plants found that all known examples of autopolyploidy in wild species have occurred in plants which are extensively or exclusively outcrossed under natural populations. Subspecies crinita, jonesii and montana were found to be obligate outcrossers in the present study and the evidence seems to indicate that their tetraploid populations are most probably of autopolyploid origin.

Most taxonomists have not given formal recognition to autopolyploids, unless there are conspicuous morphological differences between them and their diploid progenitors (Lewis, 1967). Raven (1962),

Mosquin (1967) and Small (1968) also consider morphological uniqueness to be a prerequisite for the formal recognition of autoploid races of diploid taxa. Because the autotetraploids of subspecies crinita, jonesii and montana were found to be essentially identical morphologically to their diploid parents, they should remain conspecific.

Morphological similarities between marginata and jonesii and crinita, suggest that the latter two differentiated independently from marginata (Fig. 93). The presence of both diploid and tetraploid populations suggests that jonesii and crinita have been separated from marginata for quite some time. In general jonesii occupies habitats which are more xeric than marginata. Its polyploid populations occupy even more xeric sites. The smaller leaves and dense pubescence of both crinita and jonesii might be an adaptation which would have an advantage in the drier sites.

Unlike the other taxa of Oe. caespitosa, subspecies caespitosa was probably derived from subsp. montana. Both occupy similar montane habitats and they share numerous morphological characteristics. Cytologically, the lack of chromosomal changes in the form of rings or polyploids supports this hypothesis. Caespitosa, unlike montana, is suited to habitats with a marked early summer precipitation peak (Fig. 5). It has expanded its range into drier areas, and was probably a recent invader of the northern grasslands. As this area underwent climatic fluctuations during the late Pleistocene (Dix, 1964; Wells,

1970) subspecies caespitosa expanded its range down the major river systems of Nebraska, North and South Dakota, and Wyoming.

Subspecies purpurea probably differentiated under the same climatic influences as subsp. caespitosa. However, instead of invading the lower elevations, it remained on the higher bluffs and foothills overlooking the northern Great Basin and northern plains of Montana. Its growth habit and morphology are consistent with the mode of the more primitive Oenotheras, but we see a shift to smaller capsules and flowers, and some evidence of chromosomal restructuring as evidenced by its small rings.

EVIDENCE OF INTRASPECIFIC HYBRIDIZATION

The subspecies of Oenothera caespitosa were found to be capable of exchanging genetic material in the greenhouse. Munz (1931) reported natural hybridization between subsp. montana and marginata where their ranges are contiguous. In these regions, ecological isolation is probably the most important factor keeping these entities from complete integration.

Hawkmoth pollination may be responsible for intersubspecific gene flow in Oenothera caespitosa as there appears to be no discrimination between the equivalent floral platforms of its subspecies. Evidence from this study has shown that hawkmoth pollination and gene flow may occur between taxa separated by more than twenty miles.

Long distance gene flow may be responsible for some of the morphological polymorphism present in many of the Oenothera caespitosa populations. Many herbarium specimens examined during the course of this study suggested intergradation and/or introgression among the subspecies. This evidence supports Munz's (1931) observations of natural hybridization in the field. No direct evidence of introgression and gene flow among subspecies was obtained during the present study.

Ecological differentiation, distance between populations and topographic barriers seem to be responsible for the restriction of gene flow among the subspecies of Oenothera caespitosa.

The relatively weak reproductive barriers existing between subspecies may be attributed to recent isolation and limited gene flow or to recent reassociations of previously geographically isolated populations. There has been a high degree of ecogeographic and morphological differentiation within Oenothera caespitosa without the development of reproductive barriers.

PHYLOGENETIC AND TAXONOMIC RELATIONSHIPS OF OE. EXIMIA AND OE. PSAMMOPHILA

Oenothera psammophila is probably of recent origin from Oe. caespitosa (Fig. 93). The sand dunes, to which it is endemic, were derived from lake deposits developed near Tarreton and sinks further west and the dunes are at least 4500 years old (Chadwick and Dalke, 1965).

Oenothera psammophila appears to have differentiated in response to drying trends in this area. One can imagine that in this area sand dunes may have been scattered along the shores of ephemeral lakes and that psammophila originally occupied these dunes. As the climate changed and became more arid, the lakes dried up permanently and the dunes gradually accumulated in atmospheric eddies localized by the nearby mountains (Blackwelder, 1954). Catastrophic selection may have occurred to eliminate marginal populations as Lewis (1953a, 1953b, 1962) has suggested to explain some of the patterns in Clarkia. Such selection would have probably accelerated differences in edaphic requirements and morphology of psammophila. Adaptation to the sand dunes of lake shores may have preadapted psammophila for existence on the dunes of St. Anthony.

The area of sympatry of Oe. psammophila and Oe. caespitosa subsp. marginata was carefully examined to determine the amount of interbreeding between the two taxa. These taxa were often separated by less than 200 yards. The absence of hybrids suggests that gene flow is not occurring, or that selection is very strong against the establishment of hybrids. This is to be expected since F_1 hybrids between psammophila and caespitosa in the greenhouse were found to be intermediate in morphology, and they did not inherit intact, the adapted morphological complex of psammophila which probably allowed to occupy such an extreme environment (Table XIV).

There is strong evidence to suggest that Oe. caespitosa and Oe. psammophila were ethologically isolated by hawkmoth pollinator preferences. Oenothera psammophila was found to be visited by hawkmoths of the genus Hyles, bees, and noctuid moths. Oenothera caespitosa subspecies marginata was only visited by hawkmoths of the genera Manduca and Sphinx (Table IV).

Similar examples of ethological isolation of sympatric or marginally sympatric species of plants are presented by Grant (1950), Straw (1955, 1956a, 1956b) Stebbins and Ferlan (1956), Grant and G ant (1964, 1965), Macior (1965, 1971), Breedlove (1969) and Levin (1969, 1971).

The uniqueness of Oenothera psammophila's morphology, habitat and pollination system, seems to be best interpreted at the specific level. This is in agreement with Nelson and Macbride (1916) who first recognized this taxon. At that time they stated that psammophila was "very distinct from its nearest relative," caespitosa.

In light of the foregoing evidence it seems appropriate to formally recognize Oe. psammophila. The following new combination is in order:

Oenothera psammophila (Nels. & Macbr.)

Stockhouse comb. nov.

Pachylophus psammophilus Nelson and Macbride,

Bot. Gaz. 61:32. 1916.

Oenothera caespitosa var. psammophila

(Nels. & Macbr.) Munz, Am. Jour.

Bot. 18:733. 1931.

Oenothera caespitosa subsp. montana (Nutt.)

Munz var. psammophila (Nels. & Macbr.)

Munz N. Am. Flora pt. V ser. 2:101. 1965.

Oe. eximia is probably of older origin, but, also derived from subsp. marginata (Fig. 93). The two are marginally sympatric in northern New Mexico and southern Colorado. Goeken (1969) argues that eximia should be derived from subsp. montana; but, morphological, ecological, cytological and breeding-system evidence from the present study, points to an origin from marginata.

Ecologically, Oe. eximia is much closer to marginata than it is to subsp. montana. They are also similar morphologically. Marginata, in its extreme form, a caulescent branced perennial, approximates the usual growth habit of the annual eximia. Leaf shape and pubescence are also quite similar. Cytologically the marginata x eximia hybrids were seven paired with good pollen stainability (Table XII).

The two taxa differ markedly however, in floral presentation. Flowers of Oe. caespitosa form a large horizontal landing platform which is ideally suited for hawkmoths such as Manduca spp. and Sphinx spp. (Figs. 42-45). Flowers of eximia are vertical in aspect (Fig. 15) and seem to be better adapted to smaller hawkmoths such as Hyles and to bees, much like the flowers of Oenothera psammophila.

The annual habit of Oe. eximia was probably acquired after it became edaphically isolated from marginata in southern Colorado or northern New Mexico. As the climate of this area changed during the late Pleistocene, catastrophic selection may have favored those populations which could survive the dry winters. Thus, the annual eximia appeared as an adaptive strategy for this area. Oenothera eximia appears to be closely related to Oe. caespitosa but is edaphically and possibly ethologically isolated from it. Oe. eximia is best recognized at the specific level as Asa Gray originally treated the taxon. "This is far the largest and most striking species of the section" (Gray, 1849).

The evidence indicates a direct relationship between the seven subspecies of Oenothera caespitosa. These taxa should remain conspecific. The data suggest that Oe. psammophila and Oe. eximia were derived independently from Oe. caespitosa (Fig. 93), and that they are ecogeographically and ethologically isolated from it.

PHYLOGENETIC RELATIONSHIPS OF THE REMAINING TAXA

The phylogeny of the remaining species of Pachylophus is not as clear as that of the preceeding species. Cytological data has shown that Oe. brandegeei (7II) differs by at least 3 reciprocal translocations from the model end arrangement of the Oenothera caespitosa alliance (Fig. 90). F_1 hybrids between the two were characterized by 1II + 304,

were morphologically intermediate (Table XIV), and had less than 1 per cent stainable pollen (Table XII).

Raven (1970) briefly reviewed the subgenus. Using primarily morphological data, he suggested that Oe. brandegeei and Oe. cavernae, both white flowered, self compatible, autogamous annuals, were derived independently from the Oenothera caespitosa complex as "the deserts of western America expanded and became progressively less favorable for their perennial ancestor" (Raven, 1970).

Both Oenothera cavernae and Oe. brandegeei probably differentiated from Oe. caespitosa after periods of range expansion and catastrophic contraction during and after pluvial cycles of the Pleistocene. Ancestral populations of cavernae and brandegeei which were left in virtual isolation in the southwest when habitats contracted during drought periods would have been subject to rapid restructuring and recombination in order to survive the catastrophic selection in these increasingly more xeric habitats. Catastrophic selection probably occurred to eliminate the marginal populations as in Oe. psammophila and eximia. Such a selective regime could have been accelerated by differences in edaphic requirements. They both are presently edaphically isolated from caespitosa. Cavernae occurs only on calcareous soils and brandegeei on volcanic soils.

Raven (1964a), has suggested that there may be a direct connection between edaphic endemism and catastrophic selection in dry regions.

Marginal populations, isolated by catastrophic or very rapid changes in the environment, are the ones most likely to undergo immediate and catastrophic selection, and as a result, be fixed at adaptive modes different from that of the main body of species (Lewis, 1962; Raven, 1964a).

The present study has shown that restructuring in brandegeei, and probably in cavernae as well, was in the form of reciprocal translocations, changes in compatibility, changes in edaphic requirements, and a change from the perennial to annual condition. These adaptations differ from those of the members of the Oe. caespitosa alliance. There, restructuring was primarily in the form of morphological adaptations and changes toward a more xerophytic environment with a minimum of chromosomal restructuring.

Oenothera tubifera and muelleri, the remaining white flowered perennial species of Pachylophus, are closely related (Fig. 93). Raven (1970) reports that the F_1 hybrids between these two taxa are vigorous. Unfortunately, cytological data was unavailable. Oenothera tubifera is self compatible with much smaller flowers than muelleri. Muelleri has been reported to be self compatible (Raven, 1970) but data from the population used in the present study shows it also to be self incompatible. Oenothera muelleri is probably the more primitive of the two, as evidenced by its larger flowers, large capsules with many seeds, and both self compatible and incompatible populations. Morphologically tubifera appears as a smaller, or dwarf form, of muelleri. Oenothera

tubifera with its more restricted distribution (Fig. 1), is likely derived directly from muelleri.

Cytological data from the parents and F_1 hybrids of these two taxa would certainly help clarify these relationships. Data from the present study was inconclusive, as crosses involving muelleri and the other species of Pachylophus resulted in no seed set. Mature plants of tubifera were obtained too late to be of any value in the breeding studies conducted during these studies.

Morphological data suggests that Oenothera muelleri and tubifera are more closely related to each other than either is to the other white flowered members of Pachylophus. Most likely muelleri differentiated early and the smaller flowered self compatible tubifera, was of more recent origin (Fig. 93).

The other Mexican species, Oenothera macrosceles, is similar in habit to muelleri and tubifera, but it has yellow flowers and much more narrow capsules. It is clearly not as closely related to Oe. muelleri and tubifera as they are to each other. Oenothera macrosceles has however, been hybridized with Oe. muelleri and Oe. tubifera in cultivation, but the seeds would not germinate (Raven, 1970).

Thus, it appears that Oenothera macrosceles bridges the geographic gap left by tubifera and muelleri with the white flowered species of Pachylophus in the United States. Cytological and breeding data from crosses involving these taxa would certainly help clarify these relationships.

The remaining species, Oenothera primiveris and Oe. xylocarpa are both yellow flowered. Primiveris is a polytypic annual. It has been reported to be both self incompatible (Klein, pers. comm.) and self compatible (present study). Xylocarpa is a self incompatible (Klein, pers. comm.) perennial. Cytologically, primiveris is heterogeneous, ranging from the seven paired condition to 3II plus 2 rings of 4. The only count for xylocarpa was reported to be 3II plus 2 rings of 4.

Oenothera primiveris seems to be ecologically, morphologically and cytologically more diverse than the perennial xylocarpa. The two species appear to be related to one another and to be only distantly related to the Oenothera caespitosa alliance. Klein (pers. comm.) has suggested that primiveris probably is derived from xylocarpa. Indeed the general trend in the angiosperms is from perennial to annual species (Stebbins, 1950, 1971; Cronquist, 1969; Takhtajan, 1969).

However, it is difficult to accept this origin for primiveris for several reasons. It is not common for a narrow endemic like xylocarpa, to give rise to a polytypic species like primiveris. Raven (1964a) and Lewis (1962) suggest that a more common pattern in the flowering plants for the origin of endemic species involves the restructuring of the marginal populations of polytypic species (Oe. primiveris in this case) which are apt to be growing in marginal habitats. These populations would be the ones most likely to undergo

catastrophic selection and as a result, be fixed at adaptive modes different from that of the main body of the species. Following such selection, the incipient species (xylocarpa in this case) would emerge as a narrow edaphic endemic of the sort common in the floras of California (Lewis, 1962, 1973; Raven, 1964a).

Stebbins (1952) has shown that it is not uncommon for a perennial taxon of relatively restricted extent to be traced to an annual species. Increasing specialization to xeric conditions and the readaptation of marginal populations to more mesic conditions has been shown to occur in desert species of Compositae and Leguminosae (Stebbins, 1952). Thus, it seems most plausible to derive xylocarpa from the more polytypic primiveris (Fig. 93).

In summary, the most striking feature within Pachylophus is the amount of differentiation, both morphological and ecological occurring without the establishment of strong barriers or discontinuities to gene flow especially within the Oenothera caespitosa alliance. Morphological and ecological isolation are more apparent in the other species of Pachylophus along with cytogenetic differentiation and ethological isolation.

The evidence obtained during this study supports the taxonomic treatment of Munz (1965) as revised by Raven (1970) with the addition of Oenothera eximia A. Gray and Oenothera psammophila (Nels. & Macbr.) Stockhouse.

The probable phylogeny of the ten species of Pachylophus has been summarized in its simplest form in Fig. 93.

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