THE REPRODUCTIVE BIOLOGY OF

PROBOSCIDEA LOUISIANICA

(MARTYNIACEAE)

by

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THE REPRODUCTIVE BIOLOGY OF

PROBOSCIDEA LOUISIANICA

(MARTYNIACEAE)

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PREFACE

The reproductive biology of Proboscidea louisianica is investigated with special emphasis on the insect-plant interrelationship. This study included only one flowering season in only a small part of the plant's range. In order to more accurately elucidate the insect-plant interrelationship much more work is needed throughout Proboscidea louisianica's range.

I wish to thank Dr. Ronald J. Tyrl, my thesis adviser, for his time and effort throughout my project. Appreciation is also extended to Dr. William A. Drew and Dr. James K. McPherson for advice and criticism throughout the course of this study and during the preparation of this manuscript.

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INTRODUCTION

Proboscidea louisianica (Mill.) Thellung, found in temperate North America, is the most widely distributed representative of the Martyniaceae, chiefly a tropical and subtropical family native to the warm parts of the New World. It can be found growing in disturbed soils and waste places from West Virginia to Illinois and Minnesota and southward to Georgia and Mexico. The fruits with their vicious claw-like appendages give the plant its common name, Devil's Claw (Mayberry 1947). Devil's Claw is an erect or prostrate freely branched summer annual which grows 3-8 dm tall. The entire plant is covered with viscid, glandular hairs whose secretions give the plant a fetid odor. The leaves are cordate with crisped edges, and are opposite near the base, but become alternate toward the apex of the plant. The strongly scented flowers are borne in racemes of 8-20 flowers at the summit of the stems and branches. The lavender, pink, or almost white flowers have yellowish and purplish mottling inside the throat. The corolla is 3-6 cm in length and bell-shaped with five lobes forming two lips (Radford 1968, Rickett 1966, Hevly 1970) (Fig. 1).

Sexual reproduction in the Martyniaceae is somewhat unusual. The stigma is composed of two, flat, sensitive lobes which rapidly close when touched (Fig. 1). The lobes reopen after stimulation provided that no pollen has been placed on the stigmatic surface. However, when compatible pollen touches the stigmatic surface the lobes generally

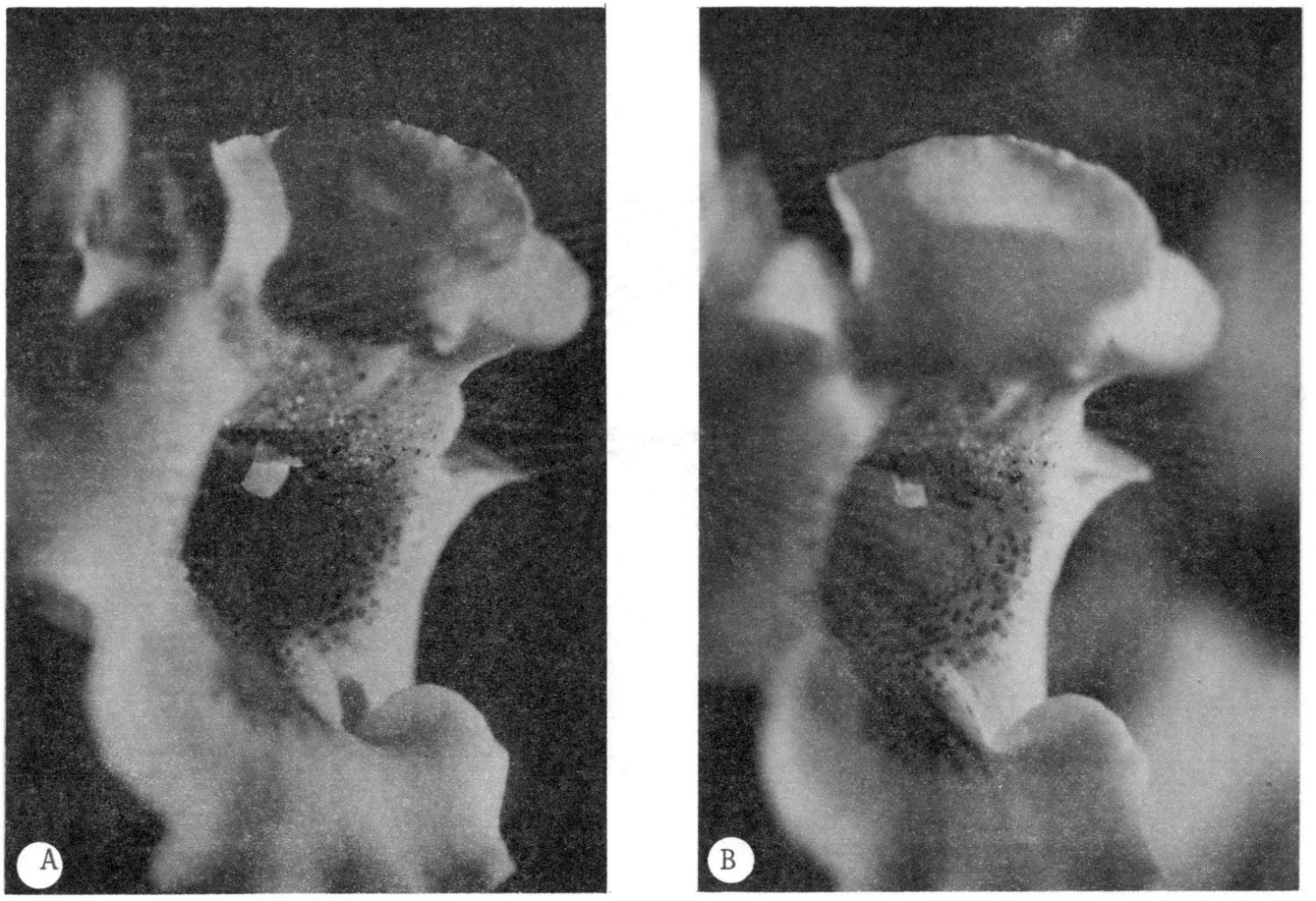

Figure 1. Flower of Proboscidea louisianica with Sensitive Stigma Visible at the Top of the Corolla Tube. A. Open Stigma B. Closed Stigma

remain closed. The sensitive stigma of Proboscidea louisianica has been superficially investigated by Anderson (1922) and Thieret (1976) and both observed that the pollinators caused the closing of the stigma as they entered the flower but before contact with the anthers occurred. These observations suggested that the stigma functions to decrease the possibility of self-pollination. Additionally, Thieret suggested that self-pollination was "fruitless" since his experiments indicated that P. louisianica was not self-compatible. The sensitive stigma and the compatibility among closely related annual species in the genus Proboscidea (Hevly 1976) suggest that the reproductive biology of P. louisianica is unusual and worthy of further study. Therefore, a detailed study of P. louisianica was undertaken in order to elucidate its reproductive biology.

In the summer of 1976, five populations of Proboscidea louisianica were located on the north shore of Lake Texoma in Marshall County, Oklahoma (Fig. 2; Table I). Thieret (1976) observed plants in the same general area in 1973. In the past this region was covered by alternating tallgrass prairies and blackjack-postoak forests generally described as "The Cross Timbers". The soils are fine sandy loam soils of the Miller series formed during the Upper and Lower Cretaceous (Bennett 1912). At present, row crops and pastures dominate the landscape. The populations, occurring in overgrazed pastures and at the edge of fields, are typical habitats for P. louisianica.

Collected insects have been deposited in the Oklahoma State University Entomological Museum and the Snow Entomological Museum at the University of Kansas. Voucher speciemens of P. louisianica have been placed in the Oklahoma State University Herbarium. In each

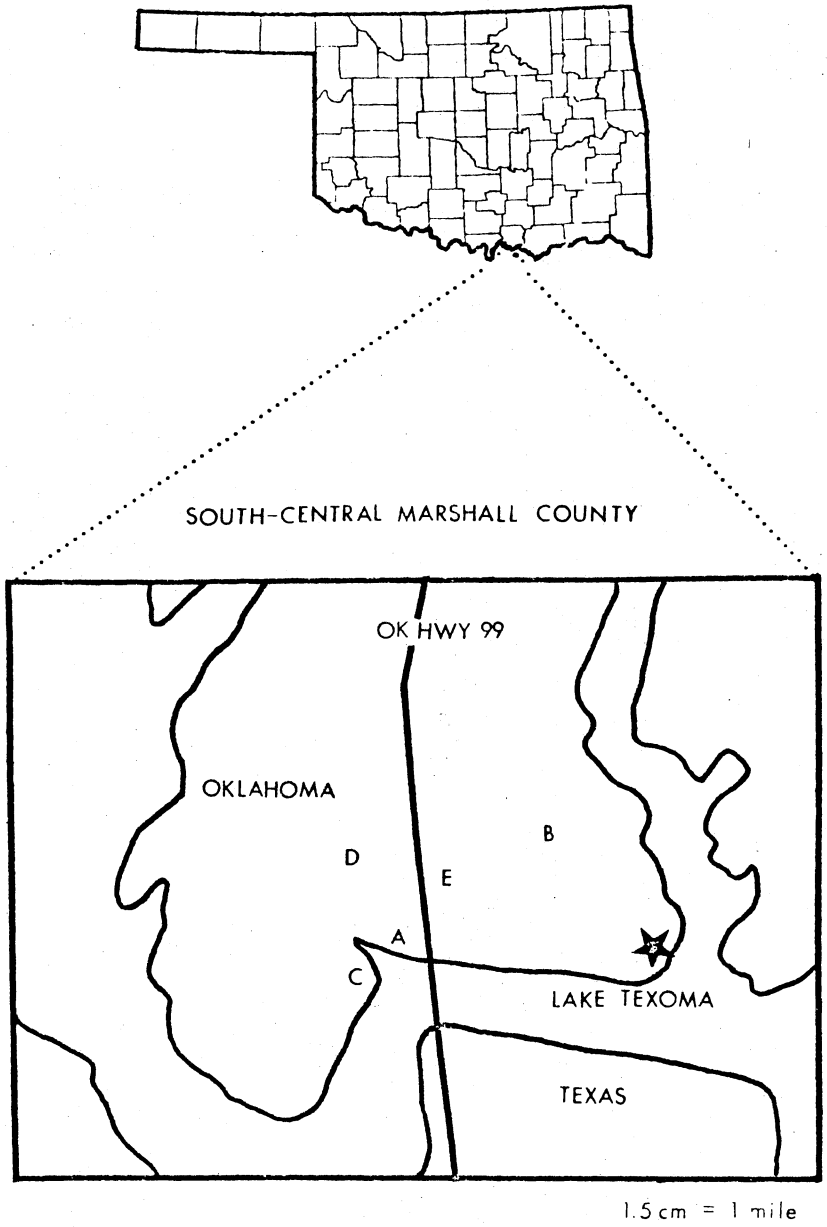

Figure 2. Populations of Proboscidea louisianica Studied in the Summer of 1976. Letters Refer to Population Locations Cited in Table I. Star Designates Location of the University of Oklahoma Biological Station.

TABLE I

LOCATION AND SITE DESCRIPTION FOR THE FIVE POPULATIONS OF *PROBOSCIDEA LOUISIANICA* SELECTED FOR STUDY

Population	Approximate number of plants	Location	Site Description
A	150	R4E, T8S, North central portion of Section 12	Sandy soil. Abandoned cornfield.
B	10-20	R5E, T8S, Northeast corner of Section 6	Sandy loam soil. Bermudagrass pasture.
C	150	R4E, T8S, Central portion of Section 12	Sandy soil. Bordering grain sorghum field.
D	10-20	R4E, T8S, West central portion of Section 1	Sandy loam soil. Bermudagrass pasture.
E	10-20	R4E, T8S, East central portion of Section 1	Sandy loam soil. Bermudagrass pasture.

population, the phenology and fruit development of the plants, the insect visitors and their behavior, and the breeding system was examined. Interpretations of the accumulated data are presented below.

PHENOLOGY

Flowering commences in late May or early June. Thieret in his 1976 paper reported numerous observations of floral development and structure. Additional observations in this study present a clear picture of phenological patterns. After a bud reaches approximately 1.5 cm in length, development proceeds rapidly until the corolla reaches a length of 3-6 cm the day before opening. The time of opening is variable usually occurring before noon, but flowers can be found opening at any hour of the day. This observation is at variance with Thieret's (1976) who reports that flowers generally open in the afternoon usually about 6:00 p.m. On the first day of anthesis, the corolla is pale yellow or yellowish white (color 1A2 or 1A3 according to the 1961 classification of Kornerup) while the inner lobes are sometimes tinged with pink (11A2, 13A3) shortly before dropping off.

The calyx, split ventrally to the pedicel, consists of five lobes with the upper median lobe being the longest. Two, thick, oblong bracteoles are located laterally to the calyx. Thieret (1976) reports that the bracteoles are deciduous at or even before anthesis. My observations, however, reveal that the bracteoles are shed during late anthesis. All parts of the plant except the inner surface of the corolla tube and the corolla lobes are densely covered with viscid glandular trichomes.

My observations of anther and stigma development agree with those of Anderson (1922) and Thieret (1976). The filaments remain short until just prior to anthesis at which time they elongate into the throat of the corolla, just below the stigma lobes. The style is tubular and terminates in a two-lobed stigma. The lower lobe, with an average length of 2.5 mm, is substantially longer than the upper which averages 2.1 mm long. Numerous viscid papillae cover the inner surfaces of both lobes. The stigma is exserted approximately 5 mm beyond the distal end of the anthers. At the base of the ovary is a dark green ring of cells that secretes nectar throughout anthesis. Ovules per ovary averaged 50 with a range of 36-73 (Appendix A).

To determine the time of pollen germination after deposition on the stigma, ten flowers were emasculated and bagged the night prior to opening. The next morning at 9:00 a.m., the flowers were hand-pollinated with pollen from another plant. Stigmas were collected at one-hour intervals following pollination, and fixed in a mixture of chloroform:ethanol:glacial acetic acid (6:3:1) and observed with a binocular microscope following staining with safranin O-aniline blue. The first pollen grains germinated within one hour of pollination.

Stigma receptivity was also examined. Thirty flowers were emasculated and bagged the night before opening. Beginning at 6:00 a.m. the following day, three flowers were hand-pollinated with pollen from another plant. The three stigmas were collected one hour after pollination, and fixed in the manner described above. Thereafter stigmas were collected and fixed at six-hour intervals for 54 hours, or throughout anthesis. The stigmas were stained in a one percent solution of safranin-aniline blue (1:1) and examined for germinated

pollen. Pollen tubes were observed on each stigma indicating that the stigma is receptive throughout anthesis.

At the time of dehiscence, the rather large, sticky pollen grains (average diameter, 80μ) are characterized by an exine that is thickened to form hexagonal surface patterns. Pollen fertility was examined. Five flowers from five different plants from each population were collected on the first day of anthesis. Pollen from each flower was scraped onto two slides. The grains on one slide were immediately stained with lactophenol:aniline blue and the first 200 grains observed were scored as either fertile or infertile. Darkly stained spherical pollen was scored as fertile, while pollen irregular in shape or faintly stained was scored as infertile. Percent fertility for all populations ranged from 92 to 96 with no significant difference between populations. Pollen on the second slide was placed in full sunlight on a tray for 48 hours, stained and observed as before. No significant difference was detected between the fertility of two-day old pollen and that of freshly shed grains which suggests that the pollen remains viable throughout anthesis.

To determine the number of grains per anther, buds from all populations were collected, killed and fixed in Carnoy's and then stained in Snow's stain (Snow 1963). Buds 1-1.5 cm long from five different plants were selected from each population. One anther was excised from the bud and dissected in 20 ml of tapwater to release the grains. A 0.2 ml subsample of this mixture was pipetted while the water was rapidly agitated to assure uniform dispersion of the grains. The subsample was transferred to microscope slides and the grains were counted. Any grains left in the pipette were also counted. Three

counts were made from each anther. The average was 10,396 grains per anther with a range of 9,200-13,000 (Appendix B). The number of pollen grains per ovule was 850. This ratio is low when compared to those of other entomophilous species (Pohl 1937) and may reflect the large size of the individual grains.

Pollen tube growth was examined for both selfed and outcrossed plants. On the afternoon before flower opening, 60 flowers were bagged, half of which were emasculated. The following day at 6:00 a.m., 30 flowers were manually self-pollinated while the 30 remaining emasculated flowers were pollinated with pollen from other plants. At each six-hour interval throughout flowering three selfed and three outcrossed plants were collected, killed and fixed in a 6:100 mixture of 37% commercial formalin to 70% ethanol (Chandler 1931). Using the technique described by Ramming (1973) the pollen tubes were stained with 0.005% water soluble aniline blue in a 0.15 M solution of K_2HPO_4 at pH 8.65 and the intact style and stigma squashed and observed by fluorescence microscopy. The pollen tube walls fluoresced a bright yellow-green. Pollen tubes reached the apex of the ovary in less than six hours with no observable difference between the growth of tubes in selfed and outcrossed plants.

The fruit is a one-celled capsule dehiscing loculicidally with two broad parietal placentae. Approximately two months after flowering the pericarp sloughs off and the endocarp splits from the apex to the base (Mayberry 1947) forming the two horns or claws that give the fruit its name. This bizarre fruit facilitates seed dispersal as it is readily entangled in the legs of herbivores, particularly cattle. Farmers

whose stock are tormented by the pain the fruit inflicts describe the plant as a nuisance (Gardner 1932).

INSECT VISITORS AND POLLINATION

Observations of the flowers of Proboscidea louisianica reveal adaptations for insect pollination (Baker and Hurd 1968, Faegri and van der Pijl 1971). The flowers are of the "gullet-type" and are characterized by the sexual organs positioned at the roof of the corolla so that pollen is deposited on the dorsal parts of the pollinator, i.e., nototribically and a prominent landing platform as is seen in P. louisianica. In addition, flowers of P. louisianica exhibit typical adaptations for bee pollination or melittophily as the flowers are zygomorphic, mechanically strong, possess well-hidden nectar, have nectar guides, and are odoriferous.

During the summer months of 1976, observations and collections were made of the insects visiting P. louisianica blossoms. The behavior of insects alighting on the corollas was recorded. The insects were then collected and were later pinned and labeled. Insects collected were examined for P. louisianica pollen. Every part of the insect where pollen was visible under a binocular scope was scraped, and the pollen transferred to a microscope slide. The pollen grains were then stained with safranin-aniline blue and identified. No attempt was made to quantify the amount of P. louisianica pollen in relation to other grains present. Insect visitors bearing P. louisianica pollen are listed in Table II. Other visitors including those observed and/or collected without P. louisianica pollen are discussed in Appendix C.

TABLE II

INSECT VISITORS BEARING *PROBOSCIDEA LOUISIANICA* POLLEN. CLASSIFICATION ACCORDING TO MITCHELL (1960)

Order Hymenoptera	Number of Individuals Collected	Number of Individuals Collected
Family Apidae		
Bombus p. pennsylvanicus De G.*	15	14
Family Anthophoridae		
Melissodes communis Cresson+	25	21
Xenoglossa strenua (Cresson)+	9	7
Centris lanosa Cresson+	2	2
Anthophora walshii Cresson+	1	1
Melissodes sp.+	1	1
Family Megachilidae		
Megachile montivaga Cresson$^{\Delta}$	2	2
Family Halictidae		
Lasioglossum (*Evylaeus*) sp.+	2	2

*Identified by Dr. H. E. Milliron, New Martinsville, West Virginia.
+Identified by Dr. Charles D. Michener, University of Kansas.
$^{\Delta}$Identified by Dr. T. B. Mitchell, North Carolina State University.

Because of their behavior, the frequency of visits and the number of individuals observed, two bee species are considered the major pollinators of P. louisianica in south-central Oklahoma. The first bee, Melissodes communis Cresson, was found foraging on Proboscidea at the beginning of the summer. As circumscribed by Mitchell (1960), Melissodes is a relatively large genus (>100 spp.) of moderately robust hairy bees. Members of this genus are regarded as important pollinators of native plants and crops. Laberge (1956) reports that M. communis is a highly polylectic and prefers flowers of the Fabaceae and Lamiaceae, particularly members of the genera Melilotus and Medicago. In addition, this species was collected from P. louisianica by Robertson (1928) and in south-central Oklahoma by Thieret (1976).

During the course of this study as many as five to ten M. communis females were seen foraging simultaneously in the large populations of P. louisianica. No distinctive flight pattern was observed and flower visitation appeared random. These insects also showed no apparent preference for the younger, light yellow flowers or the older pink flowers. When entering the flower, the bee lands on the lower lobe of the corolla and moves into the corolla tube. Occasionally it may turn upside down while inside the corolla so that pollen is deposited both sterno- and nototribically.

Only females, 12-16 mm long, were captured and of the 25 individuals collected, pollen of P. louisianica was found on the head, thorax, abdomen, and scopae of twenty-one. Pollen is a rich source of food, especially protein, and is used in nourishing the larvae. The bees were also observed utilizing the nectar, probably for individual maintenance (Faegri and van der Pijl 1971).

Melissodes communis was observed and collected primarily in June, July and early August. Thereafter, the bees were only rarely seen. According to Laberge (1956) M. communis is most abundant from the end of June through August, but can be collected from March to September. The abrupt decrease of M. communis cannot be explained adequately, as Proboscidea was still in full bloom and there was no substantial increase in flowering of other plants that might have attracted the bees. Melissodes communis is thus believed to be major pollinator of P. louisianica. The geographic extent of this specific relationship awaits further collections from other populations of P. louisianica throughout its range.

In June, July and early August, M. communis was the only regular visitor to Proboscidea. In early August the number of M. communis visits decreased; at the same time, workers of Bombus pennsylvanicus pennsylvanicus De G. began visiting P. louisianica. Although visiting Solanum rostratum, Monarda punctata, and Helianthus annuus which occurred among the populations of P. louisianica, this large bumblebee had not previously been observed landing on Proboscidea. It became the major pollinator throughout the remainder of Proboscidea's flowering season which ended in early September.

Bombus is a native group of annually social bees. Fertilized queens hibernate during the winter, and in the following spring start new colonies. The early broods that result from the nest-building, foraging, and egg-laying activities of each queen are workers that are small in size. These workers assume much or all of the foraging and nest-building functions (Mitchell 1960). Finally, males and queens are produced which mate and the cycle is repeated (Free and Butler 1959).

The species is distributed throughout the U.S., northern Mexico and southern Canada.

The bumblebee approaches the flower, lands on the lower lobe and moves as far into the corolla as it is able. Pollen of P. louisianica was identified on 14 of the 15 individuals being collected from the dorsal portions of the head and thorax as well as in the scopae. The bumblebees were also observed utilizing nectar. Bombus pennsylvanicus individuals were observed visiting Solanum rostratum and Monarda punctata as well as P. louisianica. This is to be expected since a colony of bumblebees is active throughout the season because of the overlapping generations of adults, and thus it is necessary for the bees to exploit a wide variety of resources as they become seasonally available (Heinrich 1976).

Solanum rostratum and Monarda punctata came into bloom later than P. louisianica; perhaps it was these species that at first attracted B. pennsylvanicus into the populations of Devil's Claw during which time the bumblebee began "minoring" (Oster and Heinrich 1976) on P. louisianica then eventually "majoring" on it. The first pollinator's decline is unexplained at this point. No acts of aggression were observed between M. communis and B. pennsylvanicus and according to Heinrich (1976) it is "unlikely that [bumblebee] colonies can seize, hold or defend territories . . . they do not give any sign of intolerance of [other species] while foraging under natural conditions."

Both putative pollinators remove almost all of the pollen from the anthers of each flower. Observations of 25 newly opened flowers on two occasions revealed that all of the flowers were visited at least once before noon of the first day of anthesis. Pollen grains were packed in

the scopae and on the head and thorax of Bombus and the head, thorax, and abdomen of Melissodes. Kraai (1962) suggests that pollen packed in the scopae does not play a role in pollination as viability is quickly diminished. The entrance of both bees into the corolla invariably stimulated the sensitive stigma to close so that its receptive surfaces were no longer exposed. The lower stigma lobe hangs down into the mouth of the corolla and comes into contact with the head, thorax, or abdomen of the entering visitor and at that time pollen from another plant is deposited on the stigma. When the pollen dusted bee exits the corolla there is little chance that self-pollination will occur, since the lobes of the stigma are closed.

THE SENSITIVE STIGMA

The sensitive stigma is not unique to the Martyniaceae but has been described from species in the Bignoniaceae (Burck 1902, Newcombe 1922), Lentibulariaceae (Hildebrand 1869), Acanthaceae (Morren 1839, Trelease 1882) and the Scrophulariaceae (Henderson 1841, Burck 1902). In most cases, an insect pollinator serves as the stimulating agent, however, Elrod (1904) observed hummingbirds fulfilling this role in Campsis radicans (L.) Seeman. The external morphologies of the stigmas are generally similar, and consist of two obovate to oblanceolate lobes which diverge at varying angles from 90^{o} to nearly 360^{o}, prior to stimulation and closing. In one genus of Acanthaceae, Strobilanthes, the sensitive stigma consists of a slender style that tapers at its apex to form the stigma. In this case, the stigma and style quickly straighten or recurve upon stimulation so as to press the stigma closely against the lower lobe (Trelease 1882).

Another common attribute of these sensitive bilobed stigmas is that the lobes reopen after stimulation, provided that no pollen has been deposited on the stigmatic surface (Newcombe 1922). However, when compatible pollen touches the stigmatic surface the lobes generally remain closed. The sensitive stigma has been generally considered to function to decrease the possibility of self-pollination. However, Burck (1902) found that the sensitive stigma of Torenia fournieri (Scrophulariaceae) functioned to increase the possibility of self-pollination. Burck (1902), Lloyd (1911), and Brown (1913) investigated the response mechanism and concluded that water withdrawal from the lobes was responsible for the stigmatic closure. Newcombe (1922) confirmed their findings and in addition, gathered evidence which suggested that an enzyme or other chemical substance in the pollen maintained closure.

Heckel first described the sensitive stigma of Proboscidea louisianica in 1874 and since that time very little work has been done investigating its role in the reproductive process. Observations from the summer of 1976, revealed a few of its characteristics. Rain or a water droplet would momentarily close the stigma, which reopened in 5-10 minutes. Sand dusted over the lobes as well as blowing on the lobes would also cause temporary closure. The stigma is sensitive to very slight stimulation and can be readily closed by pulling a human hair across the lobes. Observations concerning fatigue of the stigma agree with those of Brown (1913) and Thieret (1976). The first stimulation of the stigma at the beginning of anthesis requires 5-10 minutes to reopen. On the 5th, 6th, or 7th stimulation reopening

generally required 25-45 minutes, stimulation after that required up to two hours.

Observations during the present study suggest that the stigma of Proboscidea louisianica primarily functions to decrease the possibility of self-pollination. The stigma may also serve to physically protect the germinating pollen.

THE BREEDING SYSTEM

In order to determine the nature of the breeding system in Proboscidea louisianica, different modes of reproduction were tested using standard techniques (cf. Radford 1974). All plants in each population were numbered. Fifty flowers at approximately the same stage of development were randomly selected in each treatment. A rachis branch of either johnsongrass or grain sorghum was employed to facilitate hand pollinations. Fruit set was checked one month later. Modes of reproduction tested and the methods employed were:

Controls: In order to estimate the percent fruit set under natural conditions, flowers were marked with a piece of pink fluorescent ribbon, but otherwise undisturbed.

Anemophily: Flowers were emasculated before pollen dehiscence, nylon stocking securely tied around the blossom and adjusted so that the stigma was exposed to the wind. The nylon stocking served to exclude insects yet would not impede air-borne pollen.

Agamospermy: Flowers were emasculated while still in the bud and bagged to prevent pollen from reaching the stigma.

Intrapopulational Xenogamy: Buds were emasculated and bagged. At early anthesis stigmas were hand pollinated with the pollen from another plant in the same population and then rebagged.

Interpopulational Xenogamy: Buds in population A were emasculated and bagged. While the bagged flowers were in early anthesis flowers from population C were collected, placed in separate four-dram vials, and the pollen was transferred to the stigmas of the bagged flowers in population A. Flowers were then re-bagged. Reciprocal crosses were also made.

Natural Autogamy: Flowers were bagged while still in bud to test for natural self-fertilization.

Artificial Autogamy: Flowers were bagged while in the bud. During early anthesis, stigmas were manually self-pollinated and re-bagged.

The results of these crosses are summarized in Table III and Appendix D. As expected, wind pollination is not part of the breeding system of Proboscidea louisianica as the flowers lack the structural modifications generally associated with anemophily (Faegri and van der Pijl 1971). Proboscidea also does not appear to be agamospermous; the one fruit occurring in population A was most likely due to technique error. Twenty-five additional flowers were tested in that population, none of which set fruit.

Intrapopulational and interpopulational crosses were equally successful. There was no significant difference between these two modes of reproduction ($p > .5$). However, a significant difference was observed between these crosses and the control percent fruit set ($p < .02$), but was most likely due to the extensive predation of control flowers by lepidopteran larvae that foraged on P. louisianica throughout the summer. Bagged flowers utilized in the crossing experiments were protected somewhat from this predation.

Natural autogamy, in the absence of biotic pollinators, does not play a significant role in the reproduction of P. louisianica as indicated by the 4% fruit set. On the other hand, the taxon is

TABLE III

PERCENT FRUIT SET UNDER EXPERIMENTAL CONDITIONS.
NO SIGNIFICANT DIFFERENCES AMONG
POPULATIONS AT 5% LEVEL,
POPULATION RESULTS
POOLED

Mode of Reproduction tested for	Number of flowers	Percent fruit set
Controls	42	52
Anemophily	46	0
Agamospermy	75	1
Intrapopulational Xenogamy	47	83
Interpopulational Xenogamy	49	78
Natural Autogamy	48	4
Artificial Autogamy	47	57

self-compatible (57%) when manually self-pollinated. Hence, the previously described spatial relationship of the essential organs and the exclusion of biotic pollinators are responsible for the lack of natural selfing. In addition, there seems to be an internal isolating mechanism partially preventing self-fertilization. There was a significant difference ($p < .05$) in fruit set between artificially selfed and outcrossed plants. As previously mentioned, a comparison of pollen tube growth between selfed and outcrossed plants did not reveal any differences. There was no distinguishable difference in the autogamous and xenogamous fruits either during development or in the number of seeds set per fruit ($p > .5$) (Table IV). Furthermore, there was no significant difference ($p > .5$) in the percent germination of the seeds from selfed or outcrossed fruits (Table V). It is suggested that the barrier is prezygotic, perhaps a failure of the pollen tubes to penetrate the embryo sac.

Cytological observations of Proboscidea are limited. Buds were collected, killed and fixed in chloroform:95% ethanol:glacial acetic acid (6:3:1), stained in Snow's and squashed in Hoyer's medium (Radford 1974). Meiotic counts were made from microsporophytes. Counts agree with those of Martini (1939) and Perry (1942) who reported a diploid number of 30. There are 15 pairs of chromosomes at meiotic Metaphase I. Meiotic Anaphase I was observed ($n = 15$). Cytokinesis is post-meiotic.

SUMMARY

A detailed study of five populations of Proboscidea louisianica in south-central Oklahoma was undertaken to determine its breeding system, phenological patterns and principal pollinators. Major findings are

TABLE IV

SEEDS PER FRUIT IN SELFED VERSUS CROSSED FRUITS FROM POPULATIONS A AND C

	Population		
	A	C	A & C
Selfed			
Number of fruit	4	10	14
Number of seeds	249	427	676
Average number of seeds/fruit	62.25	42.7	46.8
Crossed			
Number of fruit	5	15	20
Number of seeds	266	636	902
Average number of seeds/fruit	53.25	42.4	45.0

TABLE V

PERCENT GERMINATION OF SELFED VERSUS CROSSED SEEDS FROM POPULATIONS A AND C*

	Population		
Selfed	A	C	A & C
Number of seeds	249	427	676
% germination	57	6	25
Crossed			
Number of seeds	266	636	902
% germination	40	8	18

*Normal fruit development requires approximately eight weeks. Germination of seeds from eight week old fruits or older is 75%. Fruits of populations A and C were collected early (six and three weeks respectively) because of extensive rodent and insect predation.

that:

1. Proboscidea louisianica is an outcrosser capable of autogamy. The sensitive bilobed stigma is the mechanism that facilitates xenogamy. A pre-zygotic barrier to self-fertilization is hypothesized to exist.

2. Of the eight insect taxa utilizing pollen of P. louisianica two, Melissodes communis and Bombus pennsylvanicus pennsylvanicus, are considered major pollinators.

Possessing attributes favoring both genecologic flexibility and fitness, Proboscidea louisianica is adapted for dispersal and occurrence in disturbed habitats. This is a common characteristic of autogamous plants as Stebbins (1958) demonstrated. It is conceivable that in earlier times before fenced rangeland, there was considerable long-distance dispersal of the Devil's Claw fruits by large herbivores. At present, the fruits of P. louisianica are thought to be dispersed by certain agricultural practices (Gardner 1932). Self-compatibility makes it possible for a single plant to reproduce and start a population.

Perpetual self-fertilization does have its advantages. Continued inbreeding tends to reduce heterozygosity, recombination, variability, and therefore the evolutionary potential of a species. On the other hand, outcrossing promotes genetic recombination and thus genetic diversity which is likely to lead to ecologic diversity. Therefore, a plant such as Proboscidea louisianica whose reproductive mechanisms encompass both cross- and self-fertilization is likely to be successful in invading new habitats.

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APPENDIX A

OVULES PER OVARY PER POPULATION OF *PROBOSCIDEA LOUISIANICA*. NO SIGNIFICANT INTERPOPULATIONAL DIFFERENCES (P = .05) AS DETERMINED BY TUKEY'S *W*-PROCEDURE (STEEL AND TORRIE, 1960)

	Population				
Ovary	A	B	C	D	E
1	57	44	54	42	44
2	68	42	61	58	47
3	55	40	36	47	42
4	40	52	70	34	53
5	43	38	73	45	43
Average	52.6	43.2	58.8	45.2	45.8

APPENDIX B

POLLEN GRAINS PER ANTHER* PER POPULATION OF *PROBOSCIDEA LOUISIANICA* AS COUNTED BY A DILUTION METHOD. NO SIGNIFICANT INTERPOPULATIONAL DIFFERENCES (P = .05)

Plant	Population A	B	C	D	E
1	12,767*	7,867	11,033	9,100	12,733
2	9,433	10,933	10,400	12,100	8,533
3	9,200	8,767	11,567	8,633	12,000
4	10,367	9,367	9,000	10,967	12,933
5	8,567	9,733	8,600	11,533	13,000
Average	10,069	9,333	10,120	10,467	11,848

*Average of three samples.

APPENDIX C

OTHER VISITORS TO *PROBOSCIDEA LOUISIANICA*

Potential Pollinators

Centris lanosa Cresson is a large bee approximately 11 mm in length that ranges from Oklahoma and Texas to Florida (Mitchell 1960). Michener (1977) considers this species "rare". *P. louisianica* pollen made up the majority of pollen found on the insect. Pollen was deposited nototribically and was found in the scopae. The bee was only observed and collected on one day during the observation period. Another species in the genus, *C. subhyalina* Fox, has been collected from *P. louisianica* in the same vicinity as the present study (Thieret 1976). A digger bee, *Anthophora walshii* Cresson was collected on one occasion. Pollen of *P. louisianica* was identified from its face and ventral surfaces but was not the only plant represented. This bee entered the corolla tube on its lower lobe. In order for the pollen to have been deposited sternotribically, the bee must have turned upside down while inside the corolla, a behavior pattern also observed for other visitors. The bee is large, approximately 14-16 mm in length, and ranges from Kansas and Nebraska east to the New England states (Mitchell 1960). *Anthophora occidentalis* Cresson is another member of the genus that was observed by Thieret (1976) to pollinate

P. louisianica. Anthophora walshii was observed and collected only on a single occasion.

One species of leaf-cutting bee, Megachile montivaga Cresson, was observed and collected on two occasions. This genus of bee is unique in that the females of pollen collecting species have the pollen brushes on the ventral side of the abdomen rather than on the hind legs (Borror and DeLong 1971). Megachile montivaga is widespread across the continent and is limited to the Nearctic region (Mitchell 1977).

Individuals in the genus Lasioglossum (Evylaeus) sp. were collected on two occasions. Thieret (1976) also collected bees from this genus on Devil's Claw in 1973. Xenoglossa strenua (Cresson) was collected on three occasions. Pollen of P. louisianica was deposited both noto- and sternotribically. This species is a common visitor to squash (Cucurbita). Mitchell (1960) reports that they are primarily matinal in their flight and "females can rarely be collected more than an hour or two after sunrise." However, the individuals in this study were collected as late as 7:00 p.m. and were frequently collected during the late morning from 10:30 to 11:30 a.m. Of the above mentioned insects all individuals collected were females, except for one Xenoglossa strenua male collected late one evening. In addition, these bees are not considered as major pollinators of P. louisianica primarily because they were observed so infrequently.

Other Visitors

Butterflies in the family Pieridae were observed occasionally visiting Proboscidea louisianica, perhaps utilizing the abundant

nectar. A Syrphid fly (Diptera:Syrphidae) was frequently seen alighting on the bright yellow spot on the lower corolla lobe, and seemed to be utilizing the secretions from the glands located there. Only on one occasion was a Syrphid fly observed to move further into the corolla tube. Contact was not made with either the stigma or the anthers. On two occasions, a hummingbird was observed hovering before and darting among the flowers of P. louisianica. A parasitic hymenopteran in the family Braconidae was continually present among and around P. louisianica foliage but was only rarely seen to alight, and then only on the leaves. At any time during the flowering season, many small insects (thrips, fruit flies, etc.) were stuck in the viscid glandular hairs on all portions of the plants, perhaps attracted by the odor of the plant or blown there by the wind. Of these visitors there was no indication that any one played a role in the pollination of Proboscidea louisianica.

APPENDIX D

PERCENT FRUIT SET UNDER EXPERIMENTAL CONDITIONS
PER POPULATION OF PROBOSCIDEA LOUISIANICA

Mode of Reproduction Tested for	Population				
	A	B	C	D	E
Control	50 (20)*	-	54 (22)	-	-
Wind Pollination	0 (22)	-	0 (24)	-	-
Apomixis	2 (50)	-	0 (25)	-	-
Interpopulational Crosses					
A pollen to C stigma	63 (24)	-	-	-	-
C pollen to A stigma	-	-	92 (25)	-	-
Intrapopulational Crosses					
within A	88 (16)	-	-	-	-
within B	-	75 (4)	-	-	-
within C	-	-	89 (18)	-	-
within D	-	-	-	75 (4)	-
within E	-	-	-	-	80 (5)
Natural Selfing	4 (23)	-	4 (25)	-	-
Artificial Selfing					
within A	58 (19)	-	-	-	-
within B	-	50 (4)	-	-	-
within C	-	-	56 (16)	-	-
within D	-	-	-	60 (5)	-
within E	-	-	-	-	66 (3)

*The number of flowers tested is in parentheses.

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