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increase in proportion of *Dodonaea* to Pine with depth and time represents an actual differential migration of the pollen.

Migration, however, is definitely not unlimited, but appears to be virtually restricted to the less compacted layers of the Sphagnum bog through which the pollen grains penetrated at the time of their original deposition from the atmosphere.

The profile taken one year after pollen dispersal indicates changes in the spatial range of the tagged pollen that are a product not only of migration but also of compression of the Sphagnum mat.

We hope that clues to the destiny of the introduced pollen and the relative significance of such compacting factors as rain and the winter snow cover coupled with structural weakening of the organic material due to decay, will be suggested by continued periodical analysis.

The annual introduction of different pollen types should provide more critical information from this Sphagnum area.

In connection with these studies, it has become apparent to us that the artificial introduction of tagged pollen is a research tool which contributes worthwhile information not only concerning the pollen itself but also the behavior of sediments by suggesting causes and effects of compression, local rates of sedimentation, the influence of climate on sediments, and the effectiveness of sampling methods and equipment.

THE CYTOLOGY OF CALTHA L.

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INTRODUCTION

Caltha L. is a genus including approximately 16 species, distributed in wet, marshy areas, in arctic and north temperate regions. The genus is assigned to the Helleborus tribe of the Buttercup Family, *Ranunculaceae* Juss. Two species are native to Minnesota, *Caltha palustris* L., the common marsh marigold, and *Caltha natans* Pall., a rare aquatic plant collected only in relatively few localities in the state. The only specimens from Minnesota in the University Herbarium document collections in the Tower, Lake Vermillion, Deep Lake, Trout River areas of St. Louis County. *Caltha natans* is also found locally in moist habitats from northern Wisconsin to Alaska and in northern Asia. *C. palustris,* on the other hand, is widely distributed throughout upper North America and Eurasia.

The new information summarized here is based primarily on analysis of *C. natans,* which has not received previous cytological attention. In the interest of obtaining data which can be used for further comparative study throughout the genus, observations have been made utilizing new material of *C. palustris.*

We are indebted to Dr. Olga Lakela of the Duluth Branch of the

University of Minnesota who collected the living material of *C. natans* used in this study at Trout River, north of Lake Vermillion (Lakela, No. 17166). The sample of *C. palustris* was collected by us at Tenmile Lake in Cass County north of Hackensack, Minnesota.

Figure 1 illustrates these two species. *Caltha palustris* is the larger of the two, with relatively large, showy yellow flowers, the showy character being due to the petal-like sepals. The stamens are relatively numerous and conspicuous in this species. *Caltha natans* is a much smaller plant, with small white flowers having relatively few stamens.

MATERIALS AND METHODS

Plants of both species were grown in the greenhouse. Actively growing root tips were killed routinely and fixed in freshly-prepared and chilled Farmer's fixative (3 parts absolute ethanol: 1 part pro-

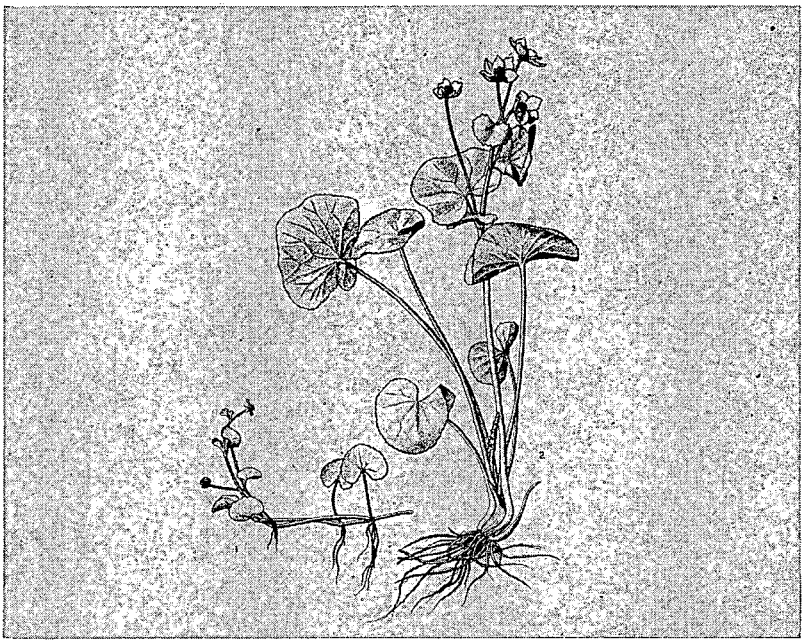

Figure 1. Habit sketches of the two species of *Caltha* considered in this study. 1. (left) *C. natans,* a small plant with inconspicuous white flowers. 2. (right) *C. palustris,* a relatively large plant with showy yellow flowers.

pionic acid). For comparison, root tips were also prepared using Jacobsen's (1954) fixative. After a suitable fixation period the material was stored at 4° C. Microscopical preparations were made in aceto-carmine according to Warmke's (1935) method. The slides were sealed with dilute "Turtox" ringing cement. Such slides remained in usable condition for several days at room temperature.

In the analysis of chromosomal morphology, camera lucida studies were made using a Leitz apochromatic objective (90X, N. A. 1.32) and a 12X compensating ocular. Accurate thread models of each of the chromosomes were constructed from the camera lucida drawings prepared for each cell. Using the kinetochore as a reference point, basic chromosome maps were developed to summarize the chromosome morphology of *C. natans* and *C. palustris*. This procedure provided the basis for exact comparison of the variation within the species as well as between the two species.

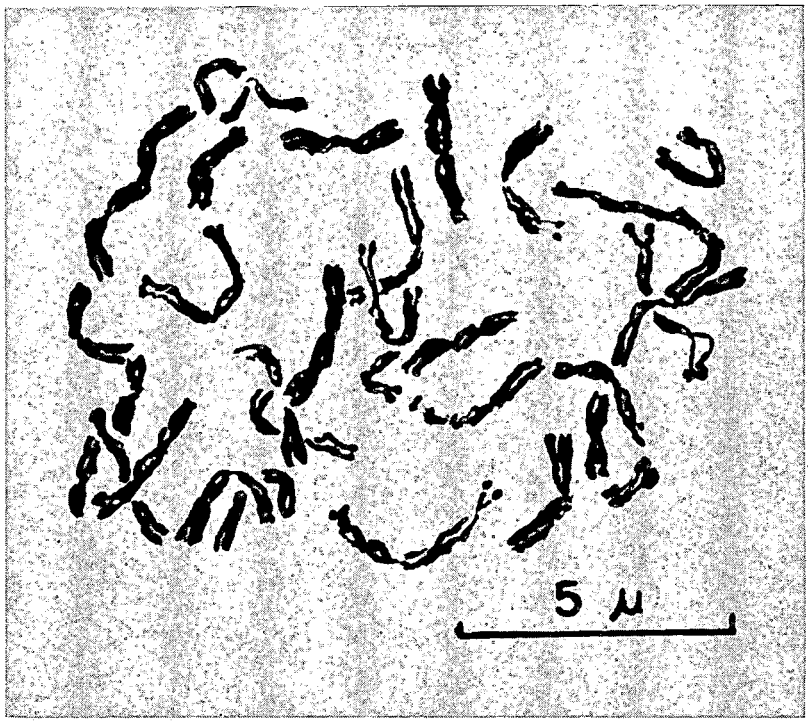

Figure 2. Camera lucida drawing of a somatic chromosome complement of *Caltha natans* (2 n = 32).

Mature pollen grains were collected from living material as well as from herbarium specimens. The mounting media used were: lactic acid (85%); lactic acid with Beibrich scarlet (1 drop Biebrich scarlet solution—0.5 gm. Biebrich scarlet in 100 cc. distilled water plus 2 drops concentrated H_2SO_4—to 15 cc. lactic acid); Belling's acetocarmine: and Calberla's solution. Living pollen grains were mounted directly in the mounting medium. Anthers from herbarium specimens were softened and pollen grains isolated in hot 45% acetic

acid. This reagent was followed by the desired mounting medium and the mounts sealed with ringing cement.

OBSERVATIONS

Figure 2 illustrates the somatic chromosomes of *Caltha natans* as we found them in root tip squash preparations. Careful camera lucida analysis revealed a complement of 32 chromosomes. Most of the chromosomes have a median or almost median kinetochore or centromere (*cf.* Figures 2 & 3). Chromatids are in evidence in the prometaphase and metaphase cells which we have analyzed. Satellite structures are

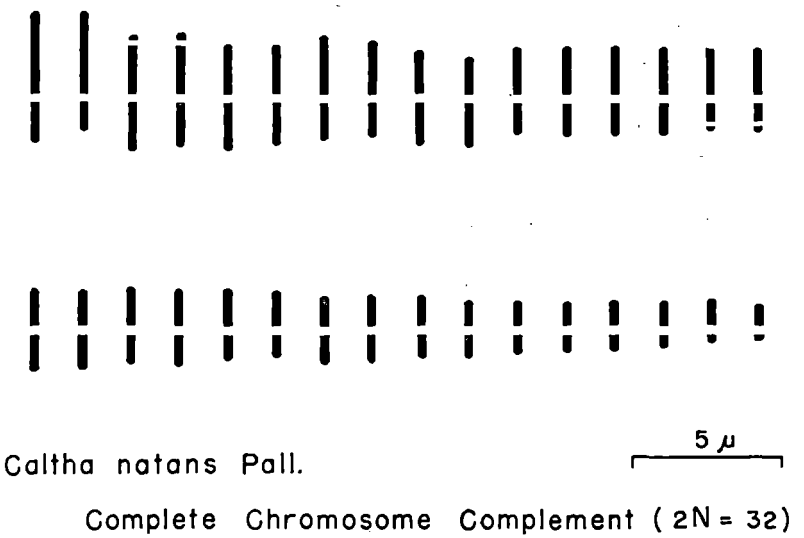

Figure 3. The chromosomes of the somatic complement arranged according to the relative position of the centromere. Satellite structures are visible in four chromosomes of the complement.

visible in four chromosomes of the complement. The chromosome maps which have been prepared from the previous analysis are shown in Figure 3. In this illustration the 32 chromosomes of the somatic complement have been arranged according to the relative position of the centromere. The longest chromosome presented in this analysis is about 4μ in length, while the shortest chromosome in the complement measures about 1μ. The average chromosomal length for the total complement is 2.5μ . It is evident that most of the kinetochores are essentially median although the chromosomes at either end of the range illustrate a sub-terminal kinetochore location.

In our routine acetocarmine squash preparations of *Caltha palustris* the average chromosomal length is 10μ for this species. The 32 chromosomes range from 3 μ to 15 μ in length. The length of the total com-

plement is ca. 313 μ. The average length of the total complement of *C. natans,* in contrast, is 86 μ.

Jacobsen's lactic acid-ethanol fixative applied to our material as a supplementary method provided well-spread cells frequently showing the kinetochore and secondary constrictions with considerable clarity. With this method, we observed a total length of 88 μ for the complete complement of *C. natans.*

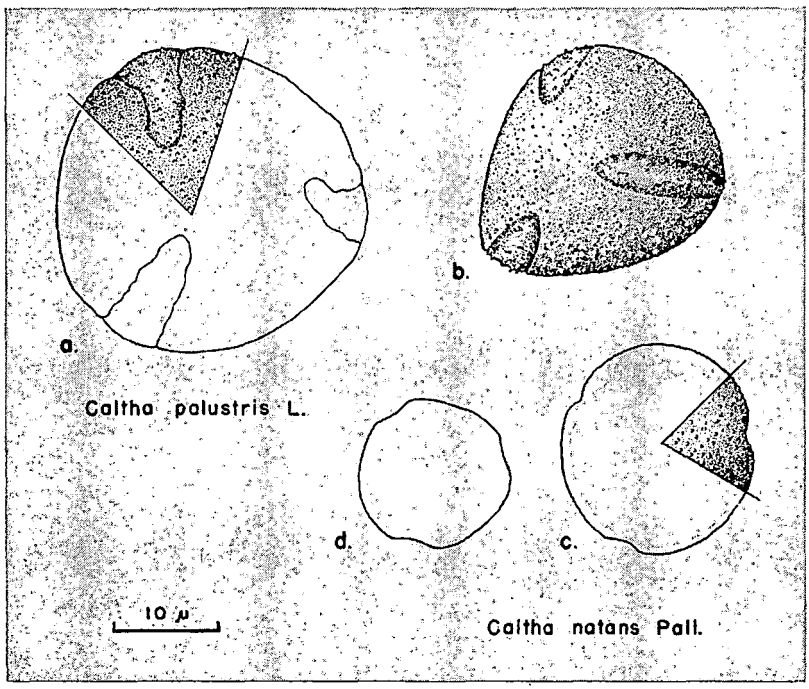

Figure 4. Camera lucida drawings of unstained, expanded pollen grains mounted in lactic acid. The drawings represent polar views. a. *Caltha palustris* (Dahl and Rowley, No. 374; Tenmile Lake, Minn.) b. *C natans* (Lakela, No. 5058; Foxboro, Wisc.) c. and d. *C. natans* (Lakela No. 17166; Trout River, Minn.).

Pollen grains of the two species are illustrated in Figure 4. *Caltha palustris* possesses a conspicuously larger pollen grain having an average diameter of 28 μ (range, 27-29 μ) while pollen of *C. natans* averages 19 μ (range, 15-25 μ) in diameter.

In individuals from two areas, however—the living plant from Trout River, Minnesota, and material from Foxboro, Wisconsin (University of Minnesota Herbarium #373545)—a 40% incidence of smaller, inviable pollen grains was observed (*cf.* Fig. 4d). These small pollen grains, which were considered to be inviable on the basis of

morphological characters, are 12 μ to 15 μ in diameter. The herbarium collections from other localities (Lake Vermillion, Tower, and Gilbert) show virtually no production of these inviable pollen grains.

The acetocarmine preparations indicate that the mature pollen grains of the two species of Caltha are binucleate at the time of anthesis.

The detailed character of the pollen grain surface is basically similar in these two species. In *Caltha natans* the exine is minutely papillate-verrucate. The most distinct of the papillae are scattered at intervals varying from 0.5-1.7 μ. The oblate pollen grains are typically tricolpate. The furrow is variable in its length (cf. Fig. 4b & c). The aperture membranes are conspicuously ornamented with scattered plaques of exine which in profile resemble papillae. It is of interest that these plaques on the furrow membranes are the most prominent sculptural elements of the pollen wall.

In *C. palustris,* the three furrows have a relatively greater length and are more conspicuous than in *C. natans.* Wodehouse (1936) has provided an excellent, illustrated analysis of *C. palustris* pollen.

DISCUSSION

Among the most significant contributions to the cytology of the *Ranunculaceae* are studies by: Hocquette, 1922; Langlet, 1927; Lewitsky, 1931; and Gregory, 1941. Langlet (1927 and 1932) suggested that within the family there were two cytological types. He designated those species having large chromosomes the Ranunculus type in contrast to another group, the Thalictrum type having small chromosomes. Gregory has followed this suggestion in presenting a diagrammatic summary of the chromosome types within the *Ranunculaceae.* His generalized treatment is perhaps the most thought provoking of the various publications on the cytology of this family. In his summary diagrams Gregory has presented the chromosome complements which he regards as typical for each of many genera within the *Ranunculaceae.* In this summary the genus *Caltha* has been assigned to the subdivision characterized by the large Ranunculus type chromosomes, typical of *Caltha palustris.* This generalization does not apply well to the chromosomal morphology we have recorded for *Caltha natans. Caltha natans* has, in contrast, rather small, slender chromosomes which are intermediate between the Ranunculus type of chromosome and the Thalictrum type of chromosome. Langlet (1932) demonstrated that *Caltha leptosepala* DC. also possesses relatively slender and small chromosomes. The accumulating evidence indicates that the chromosomal cytology of *Caltha natans* allies it with the white flowered western forms such as *C. leptosepala.*

Despite the small sample which has been analyzed, three or possibly four species, there is evidence for significant variation in the cytology of the genus *Caltha.* We feel that it is likely that further analysis may provide the basis for revised assignments within the genus.

SUMMARY

1. This study describes the chromosomal cytology and pollen grain morphology of *Caltha natans* and *C. palustris.*

2. The complement of *C. natans* is made up of 32 chromosomes. Chromosomal maps indicate that most of the chromosomes have a median or submedian kinetochore and range in length from 1 μ to 4 μ. The average chromosomal length is 2.5 μ. *C. natans* has not received previous cytological attention.

3. In contrast with *C. palustris* the chromosomes of *C. natans* are relatively small and slender. The 32 chromosomes of *C. palustris* vary in length from 3 to 15 μ with an average length of 10 μ.

4. The pollen grains are tricolpate and have a minutely verrucate-papillate exine with relatively conspicuous plaques ornamenting the germinal furrows. The pollen of *C. natans* is smaller (average 19 μ) than *C. palustris* (average 28 μ).

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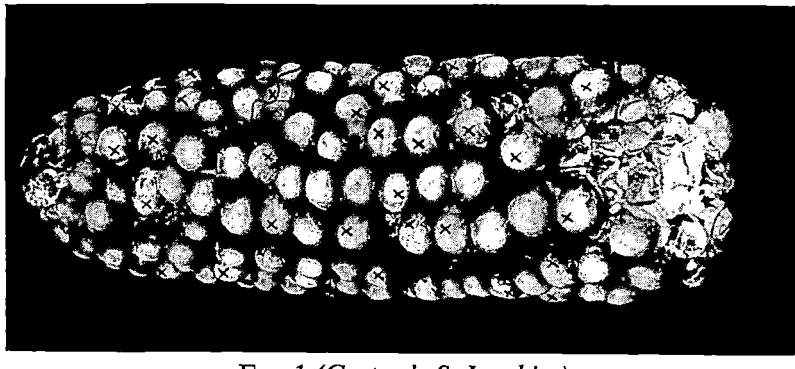

FIG. 1 *(Gertrude S. Joachim)*