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THE UNIVERSITY OF OTTAWA

A Biosystematic study of the *Polygonum lapathifolium* L. complex in North America

by

Laurie Lynn Consaul

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ABSTRACT

The Polygonum lapathifolium complex is often divided into two species in North America: P. lapathifolium L., considered partly native and partly introduced, and P. scabrum Moench, regarded as introduced from Europe. With no unique morphological character separating them, these species are based upon combinations of characters. Many of these characters are phenotypically plastic; for example, almost complete convergence of growth habit occurs under cultivation among the taxa in many populations. Nevertheless, from consideration of fruit characters and life history patterns, most of the populations studied here can be assigned to one of the two species. A strong relationship between identification as P. scabrum and non-dormancy of seeds is one important argument for taxon recognition. Yet the existence of many intermediates, the absence of characteristic flavonoid compounds, and the very low genetic variability in allozymes (only 3 of 22 loci showing variation) provides evidence that taxon recognition should ultimately be at the subspecies level. Two widely variable subspecies can be recognized: P. lapathifolium ssp. lapathifolium and P. lapathifolium ssp. tomentosum (= P. scabrum). The low variation in European populations (5 of 22 loci are polymorphic), may rule out the possibility that the low levels of variation in North America are due exclusively to founder effects.

One allele of the enzyme LAP seems to be diagnostic for North American P. lapathifolium. Found in 25 of the 27 North American populations identified as P. lapathifolium, it was not found in any of

the 13 European or Japanese populations studied nor the 15 populations of P. scabrum. The alternate allele was found in these populations, plus the remaining two populations of North American P. lapathifolium. This provides evidence for the existence of native North American races, as does the fact that some populations are found in areas not greatly disturbed by man. Nevertheless, the occasional presence of heterozygotes of this enzyme (Lap-$1^{57/53}$) supports the existence of a single variable species.

RESUME

Le complexe Polygonum lapathifolium est souvent divisé en 2 espèces: P. lapathifolium L. est une espèce considerée en partie indigène et en partie introduite alors que P. scabrum a vraisemblablement été introduite d'Europe. On ne peut distinguer ces deux espèces selon un trait unique, mais on doit plutôt utiliser des combinaisons de caractères. Plusieurs de ces caractères sont influences par les conditions environmentales; par exemple, chez plusieurs populations, la forme de croissance de ces taxons démontre une convergeance presque complète en culture. Toutefois, en tenant compte des caractères du fruit et du mode de développement, il est possible d'attribuer l'une ou l'autre de ces identités a la plupart des populations. Il existe une correspondance très étroite entre l'identification de P. scabrum et l'absence de periode de latence des achènes. Pourtant, la presence de plusieurs individus intermédiaires, l'absence de composés flavonoides caractéristiques et le taux peu élevé de variabilité génétique au niveau des allozymes (seulement 3 sur 22 sont variables) suggèrent que l'identification devrait se faire non pas au niveau de l'espèce, mais de la sous-espèce. On peut distinguer deux sous-espèces: P. lapathifolium ssp. lapathifolium and ssp. tomentosum (Schrank) Danser. Bien que possiblement valide comme taxon en Europe, P. lapathifolium var. salicifolium ne peut être accepté comme une varieté en Amérique du Nord. Les taux peu élevés de variation chez les populations européennes (5 allozymes sur 22 sont polymorphiques) tendent a éliminer la possibilité que le manque de variation constate en Amérique du Nord soit le resultat de "founder effects".

Un allèle de l'enzyme LAP semble pouvoir être utilisé comme caractère diagnostique pour les populations nord américaines de P. lapathifolium. Present chez 25 des 27 populations de P. lapathifolium en Amérique du Nord, il n'a été observé ni chez les 13 populations européenes ou japonaises, ni chez 15 populations de P. scabrum que j'ai etudiées. L'autre allèle se retrouve dans ces populations, et dans les deux autres populations de P. lapathifolium. Ceci semble appuyer la possibilité de races natives d'Amérique du Nord, d'autant plus que plusieurs de ces populations se retrouvent dans de regions peu perturbées par l'homme.

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I wish to thank my supervisor John McNeill for the opportunity to work on such an intriguing group. His enthusiasm encouraged my interest in all aspects of plant systematics, and his support continued even at VERY long distances. (I would also like to thank his car for much devoted service). My labmate Deborah Katz and I learned the satisfaction of hard work even if it meant many meals eaten out of plastic containers. The Chinese dinners will never be forgotten.

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1. INTRODUCTION

1.1. Theory

1.1.1. Taxonomy and Biosystematics of Weeds

Accurate taxonomy is essential for effective communication about living organisms. This is particularly important with economically important plants such as weeds.

McNeill (1982) gives several examples that illustrate the importance of correct classification within weed groups. These include differential resistance to 2,4-D within the Caryophyllaceae, and different ecological tolerances and resistance to 2,4-D in three species of what had originally been treated as a single species Cardaria draba (L.) Desv. in North America (Mulligan and Findlay, 1974).

Unfortunately, there is often no single 'correct' taxonomic treatment in a group of plants with complex variation (McNeill, 1982). In addition, when one encounters continuous variation over a range of species, it may be necessary to dissect a continuum to form a classification (Prentice, 1986). In such situations, it remains necessary to assign names to infraspecific taxa whenever the variation can be dissected into fairly homogeneous subsets. When, however, incongruence among data sets arises, this dissection can be difficult in practice. The example of Silene latifolia Poiret given by Prentice (1986) illustrates this.

Classification, in which taxonomic groups are recognized and delimited, is differentiated from identification, in which organisms are assigned to these classifications and named accordingly (Stace, 1980). Identification of weeds can be difficult, for several reasons, discussed in detail by McNeill (1982). First of all, one may have only propagules or young plants to examine, and the classification itself may be incomplete in characters studied. Classification with high predictive value is sought, and if classifications are based solely on a limited number of morphological characters and other characters are incongruent with the clines or groups described, the predictive value is lost. Secondly, the classification may be incomplete in that the total geographical range of the species may not be covered. As important as the choice of reliable characters is a global view of the group under study (Prentice, 1986; McNeill, 1982).

Phenotypic plasticity can also play a complicating role in the creation of classifications. It has been studied since the latter part of the last century, although until recently, it had not been allotted much attention (discussed in Morisset & Boutin, 1984). The potential for phenotypic plasticity is genetically controlled. A study of Ononis spinosa L. ssp. spinosa and O. repens L. (Morisset & Boutin, 1984) provides an excellent example of developmental and environmental plasticity that can provide clues to the evolution of character development in plants. Because of its adaptive significance, phenotypic plasticity should not simply be regarded as an "often misleading source of variation" (Bell 1957), but should play a

major role in biosystematics.

1.1.2. Numerical Taxonomy

In studying a group of plants by investigating population samples, one measures characters, whether morphological, physiological, cytological, or chemical. These character sets are then analyzed, almost always involving numerical methods, for recognition of the pattern upon which a classification can be based. This is the essence of phenetic numerical taxonomy.

Multivariate techniques are used to observe the relationships of the basic units of study - operational taxonomic units (OTUs) by considering the associations among the characters themselves as well as those among populations. Cluster analysis, ordination, and discriminant analysis all can reveal patterns resulting from shared character states.

As a result of constructing a phenetic classification a large number of common properties can be ascribed to members of a given taxon. Ideally, the states of characters not originally included in the analysis can be predicted by examining relationships within the classification. When this can be done, the classification is likely to be stable and particularly useful.

1.2. Section Persicaria

The number of species recognized in Polygonum section Persicaria varies with different taxonomic treatments. Small (1895) recognized 25 species in North America, of which 12 occurred in the northeastern

United States and northward, whereas Fernald (1950) recognized 20, and Gleason (1963) 16 species in the latter region. These different treatments are contributed to in part by discrepancies in the number of species recognized in the smartweed group that comprises the P. lapathifolium complex and the closely related species P. persicaria and P. pensylvanicum.

This group is comprised of three (Gleason, 1963) or four (Fernald, 1950; Scoggan, 1978) annual species and their varieties, and is characterized by having flowers in dense terminal racemes, and by being generally erect, non-aquatic plants, with entire ochreae an.. firm leaves.

1.2.1. Previous taxonomic studies on related groups.

Previous studies on other members of the section Persicaria have shown that several morphological characteristics are quite plastic, and the use of these characteristics in classification seems to have created problems throughout the section.

Mitchell (1968) concluded that the P. amphibium L. complex (including P. natans Eaton and P. coccineum Willd.) comprised a single species P. amphibium, with two North American varieties, var. stipulaceum Coleman (=P. natans Eaton) and var. emersum (= P. coccineum Willd.). Mitchell considered that the plasticity of characters under different environmental conditions (especially whether or not the plant was growing in water) had previously caused confusion in the classification. Fassett (1949) provides a taxonomic treatment of P. punctatum Elliott in which he describes the great

variability in many characters: size and habit of plant, denseness of the spike, shape of leaves, sheaths +/- ciliate, achenes lenticular or both trigonous and lenticular, length of calyx. Nevertheless, he considers it to be a single species and describes eight varieties in his classification. McDonald (1980) investigated the morphology, cytology and crossing compatability of the P. hydropiperoides Michaux complex, and concluded that P. hydropiperoides, P. setaceum Baldwin ex Elliott and P. hirsutum Walter were distinct, and P. opelousanum Riddell ex Small should be merged with P. hydropiperoides, not even being recognized as a variety of the latter. Characters that were discounted as not being diagnostic for species distinction between the latter two species included: leaf shape as being lanceolate to linear-lanceolate or linear-lanceolate to linear; and the achene being included in the perianth at maturity or exserted, because these characters were considered superficial. It will be seen that the use of the above mentioned characters also pose problems in plants of the P. lapathifolium complex.

1.3. The Smartweeds: P. lapathifolium, P. persicaria and P. pensylvanicum.

It became evident from the treatments in North American and European Floras that P. persicaria L. and P. pensylvanicum L. may each easily be considered a complex in themselves.

Polygonum pensylvanicum is a North American tetraploid species (counts performed here, later confirmed in more intensive study by Taylor-Lehman (in prep.)) with 2n=44 chromosomes. It is easily

distinguished from members of the P. lapathifolium complex and P. persicaria by its long stalked peduncular glands and very large, dark, shining achenes, usually with a bright pink perianth (Fernald, 1950). Experience in field collecting has confirmed the general ease of identification of these plants. This species has been divided into several varieties; Taylor-Lehman, however, revealed that some of these are not constant in cultivation.

Polygonum persicaria is an introduced species to North America (Fernald, 1950). It has been widely reported as having a chromosome number of 2n=44, with four reports of 2n=40, and 3 of 2n=22. It is easily distinguishable from the other taxa by the combination of well developed bristles or cilia on the ochreae and small, dark, shining achenes that are often trigonous. Two varieties are recognized by Fernald: var. ruderale (Salisb.) Meisn. and var. angustifolium Beckh. A closely related species recognized by Fernald is P. puritanorum Fern., which is distinguished by Fernald by most parts being smaller in size than those of P. persicaria.

The taxa referred to here as belonging to the P. lapathifolium complex (see below) include North American and European taxa that have been classified at different taxonomic levels by different authors (Table 1). Species, subspecies and varieties have been described in North America and Europe, and clarification is required.

1.3.1. The Polygonum lapathifolium complex

1.3.1.1. History of Taxonomic Treatments

Taxonomic treatments of these taxa date back to the 18th and

Table 1: Current nomenclature within the Polygonum lapathifolium complex in North American and European Floras. First name[1] is that found in Fernald (1950) and Scoggan (1979), followed by synonyms.

Source	P. lapathifolium L.[1] = ssp. lapathifolium = ssp. nodosum (Pers.) Danser = P. nodosum Pers.	P. scabrum Moench[1] [=P. lapathifolium ssp. tomentosum (Schrank)Danser =P. tomentosum Schrank
Britton (1933)	P. nodosum All forms with smaller fruit than P. lapathifolium and smaller glands	P. lapathifolium
Clapham, Tutin & Warburg (1962)	P. nodosum similar to P. lapathifolium but usually smaller & decumbent, ochreae shortly fringed, infl. rather lax, slender & +/- interrupted, acute & shortly peduncled. Perianth segments usually pink, sparsely glandular, peduncles and undersides of leaves densely dotted with yellow glands. Fruit, 2mm or slightly less.	P. lapathifolium stem usually greenish, swollen above nodes, flowers greenish-white, rarely pink. Fruit 1.5-2.5mm.
Fernald (1950)	P. lapathifolium usually with many arching to peduncled slender pink spikes (incl. P. nodosum Pers. & P. incarnatum Ell.)	P. scabrum abundant sessile glands near the summit [of stem] spikes thick, green, sepals not forming a beak at least the lower [leaves] retaining more or less flocculent white tomentum on the lower surface.

Table 1 (cont.):

Gleason (1963)	Probably both native and introduced_The most noteworthy form, possibly worthy of species rank_P. tomentosum Schrank_a form of this_ P. lapathifolium var. incanum (Roth) K. Koch_The common form_ may be distinguished when desired as P. lapathifolium var. nodosum (Raf.) Weinm.	
Hulten (1970)	(P. nodosum) -often lacks glands on the peduncle	more glandular than P. nodosum
	customary to distinguish 2 species	
Komarov (1936)	P. nodosum	P. scabrum
Ohwi (1965)	P. lapathifolium nodes swollen, leaves often red-brown when dried with minute strigose hairs spikes white or reddish.	P. scabrum closely resembling former but smaller. Achene 2-3 mm across, flowers white or reddish.
Scoggan (1978)	P. lapathifolium leaves broadest well above base, not long attenuate. Spike pink to purplish _plant often bearing sessile glands on the peduncle and lower leaf surface; calyx constricted into a thick beak overtopping the achene, this not much more than 2mm long, spike often somewhat pendulous - not over 1 cm thick.	P. scabrum spikes green (rarely purplish) to 5cm long, erect, the lateral ones mostly sessile or short stalked, floral axis copiously glandular ,achenes about 3 mm long, about equalling the mature calyx, this not constricted at the tip.
Small (1985)	P. lapathifolium racemes drooping, achenes mostly lenticular, sometimes triquetous, achene broadly oblong-ovoid flowers flesh-coloured; style 2-parted to well below middle.	(P. incarnatum) achene ovoid, flowers white-green or rose-coloured,style 2-parted to base.

Table 1 (cont.):

Webb & Chater (1964)	P. lapathifolium incl. P. nodosum Pers., P. scabrum Moench, and P. linicola Sutulov– extremely variable especially in habit, colour of foliar glands and of perianth and in indumentum. [P. nodosum Pers.] a commonly recurring variate with glabrous leaves, yellow foliar glands, red spotted stems & pink flowers in a rather lax spike, in some regions. [P. tomentosum Schrank] low habit, densely tomentous leaves & greenish-white flowers is often seen on drying mud as a weed in fields. "In both cases however, it is possible to find plants with some of these characteristics perfectly developed & others not at all and it seems doubtful whether anything is gained by giving them taxonomic rank."

19th centuries (see references in Small, 1895). These treatments plus those of Ascherson and Graebner (1913), Danser (1921, 1931) and Irigoyen and Thellung (1929) contribute to a considerable number of names describing these plants. Table 1 summarizes the currently used names in North America and Europe and the characteristics attributed to each name. The name P. lapathifolium as used by Linnaeus served merely to distinguish the complex as a whole from P. persicaria and P. pensylvanicum as illustrated by his diagnosis: Polygonum floribus pentandris semidigynous, staminibus corollae regulari aequalibus = Polygonum with pentandrous and semidigynous flowers, with stamens equal to the regular corolla. In this broad sense the name is still widely accepted and applied to the whole P. lapathifolium complex.

The next species to be described in the complex was P. tomentosum, by Schrank in 1789. Schrank did not include P. lapathifolium in his treatment of Bavarian plants, but said of his new species that it was: with entire lanceolate leaves, felted underneath, with ciliate ochreae; with uninterrupted spike; [translation of the Latin]. He also based it at least in part on a species recognized by Haller (1768), who did not adopt the Linnaean binomial system of nomenclature, citing: Polygonum foliis ovatolanceolatis, subtus tomentosis, spicis ovatis, vaginis ciliatis. Haller Hist. n. 1556. (Haller, 1768) [Polygonum with ovato-lanceolate leaves, tomentous beneath, ovate spike, ciliate ochreae].

Moench described another species, P. scabrum, in 1794: with an erect stem scabrous at the top; with ovato-lanceolate leaves tomentous and glabrous underneath; with truncate integrate ochreae; with a

cylindric spike; with hexandrous flowers and a bifid style; with a white corolla [translation of Latin] and also including the same Haller species, viz: Polygonum foliis ovatolanceolatis subtus tomentosis, spicis ovatis, vaginitis ciliatis. Hall. 1556. The name P. scabrum is applied in North America (Fernald, 1950, Scoggan, 1978) and in Japan (Ohwi, 1965) to weedy plants introduced from Europe. Confusion often occurs in the use of the names P. scabrum and P. tomentosum. Because the two names are considered to apply to the same species and because P. tomentosum is the earlier, the studies by Danser (1921, 1931) and Irigoyen and Thellung (1929) use the epithet tomentosum although Moench's broader description of P. scabrum reflects more accurately the variation encompassed by the species.

A fourth species, P. nodosum, was described by Persoon (1805). He first described P. lapathifolium with Linnaeus' description, except that he changed 'floribus pentandris' to 'floribus hexandris'. He then described his new species as: with a stem with elongate spots to the joints of the nodes, ochreae naked, spikes branched; [translation of Latin]. Britton (1933) used P. nodosum as a separate species from P. lapathifolium, and described varieties for each (including P. lapathifolium var. tomentosum Beck). He indicated that the only reliable characters separating P. nodosum from P. lapathifolium were that: in the former, the fruits are smaller, less roundish with a more ovate outline, and the glands on the peduncles and perianth are also less numerous and at times almost absent. Tutin in Clapham, Tutin and Warburg (1952) also recognized P. nodosum as a distinct species, similar to P. lapathifolium but with a lax

slender inflorescence, smaller achenes and yellow glands on the undersurface of the leaf. In this treatment the name P. lapathifolium is used for the P. scabrum/P. tomentosum kind of plant discussed earlier, whereas P. nodosum has features of plants referred to P. lapathifolium in North America.

Other authors have treated the segregate taxa at the subspecific level:

1. Danser (1921) performed extensive cultivation studies and determined that the species P. lapathifolium should be divided into four subspecies: ssp. nodosum, ssp. tomentosum, ssp. mesomorphum and ssp. leptocladum. His ssp. mesomorphum has characteristics of both ssp. nodosum and ssp. tomentosum, and is intermediate between them. Ssp. leptocladum is a quite unique subspecies. Danser raised five more names from race to subspecies in 1931 (Danser, 1931).

2. In their intensive study of P. lapathifolium in North America, Irigoyen and Thellung (1929) accomodate the variation in this species within three subspecies: ssp. nodosum, ssp. tomentosum, ssp. mesomorphum and races thereof. They adopt many of the race epithets used by Danser (1921, 1931) in his treatments of P. lapathifolium. The typical "var. lapathifolium" was not named in either of Danser's treatments nor in Irigoyen and Thellung's treatment.

3. Hégi's (1931) more recent treatment serves as an update of Danser's 1921 classification. Hégi does, however, use the autonym P. lapathifolium ssp. lapathifolium in place of ssp. nodosum, and ssp. pallidum, which he considered to be the earlier name, in place of ssp. tomentosum.

4. Fernald (1950) recognizes two varieties of P. lapathifolium in North America; the typical variety that would now be called var. lapathifolium (which, of the afore mentioned names, would probably correspond most closely to P. nodosum Pers. or P. lapathifolium ssp. nodosum (Pers.) Danser), comprising both native and introduced plants, and the other var. salicifolium Sibth., being native (per Scoggan, 1978). Fernald describes the latter variety as being small, with slender, simple to slightly branched stems 0.5-0.6 dm high; the leaves white-pubescent beneath and narrowly lanceolate to 3-10 cm long and 3-10 mm wide; "spikes 1-few, erect, 1-3.5 cm long". From Fernald's description, var. salicifolium would not correspond to any of the afore mentioned names but perhaps to a native race recognized by Irigoyen and Thellung (1929). The variety var. salicifolium was originally described by Sibthorp (1794) on the basis of plants from London, England: procumbent Persicaria maculosa, with leaves incanescent beneath; [translation of Latin]. Although used for native North American plants, it is probably actually correctly applicable to the same tomentose-leaved plants as P. tomentosum and P. scabrum.

Still other authors (e.g. Gleason, 1963) have stated that P. lapathifolium is highly variable, and that one form (P. tomentosum) "is possibly worthy of taxonomic rank". Webb and Chater (1964) in Flora Europaea noted its extreme variability, and remarked upon the probability that any pattern that may have existed has been destroyed because of the development of many autogamous lines and its widespread dissemination as a weed.

1.3.1.2. Nomenclature used in this thesis

The applicability of these names will be considered as a part of the results, but in the meantime these names will be applied as follows:

a) Plants collected in North America and keyed in Fernald (1950) to **P. lapathifolium** or to **P. scabrum** will be referred to as such. As far as possible, the variety of P. lapathifolium recognized by Fernald (**var. lapathifolium** or **var. salicifolium Sibth.**) will be indicated.

b) Plants from Europe: As seed collections, these were received under the following names: P. lapathifolium, P. lapathifolium ssp. lapathifolium, and P. lapathifolium ssp. nodosum. These will be referred to by the following names from Danser's (1921, 1931) and Hégi's (1031) treatments:

Plants received under the names P. lapathifolium and P. lapathifolium ssp. lapathifolium (having large achenes) are identified as **P. lapathifolium ssp. tomentosum** and P. lapathifolium ssp. nodosum as Hegi's **P. lapathifolium ssp. lapathifolium**.

The descriptions for these names (modified from Danser (1921) and Hégi (1931)) are as follows:

Polygonum lapathifolium L. ssp. tomentosum (Schrank) Danser.

Achene 2.5-3.5 mm long, nearly as wide. Perianth richer in chlorophyll than ssp. lapathifolium. Combination of very glandular peduncle

and cilia to 1-1.5 mm long on upper ochreae and ochreoles giving a scabrous edge.

Polygonum lapathifolium L. ssp. lapathifolium

Achene 1.5-2.2 mm long, 1-2 mm wide. Perianth rounded, closed and often beaked over the inflorescence. Inflorescence is a long, narrow, sometimes drooping raceme, terminal or at the apex of axilliary branches. May be short-peduncled axilliary inflorescences if grown in stressed environment. Stem often with red anthocyanin in ring under nodes and in spots on internodes. Little chlorophyll in fruiting perianths, which are often light to deep pink.

1.3.2. Geographical distribution

The natural range of P. pensylvanicum is restricted to North America. Polygonum persicaria is native to and widely distributed throughout the Old World and introduced to the New World; P. lapathifolium is widely spread throughout the Northern Hemisphere, extending into Indonesia, Central and South Africa and introduced in Australia and southeastern South America. It is believed to be native both in the Old and New World (Figure 1). With respect to the ranges of these plants in Canada, locations for specimens of P. lapathifolium var. lapathifolium, P. lapathifolium var. salicifolium and P. scabrum from the herbaria DAO, CAN, TRT and TRTE as keyed out in Fernald (1950), are mapped in Figure 2. Polygonum scabrum has been collected more intensely in southwestern Canada, whereas P. lapathifolium var. salicifolium has been collected farther northwest, extending to the Yukon.

Figure 1: World distribution of the P. lapathifolium complex, compiled by Hultén (1971):
Stippled area=common,
Solid line=surrounds range when only districts or other general areas known,
Dotted line=tentative border of range,
Dots=from specific locations. Range outside of the map area indicated by place names on the map.

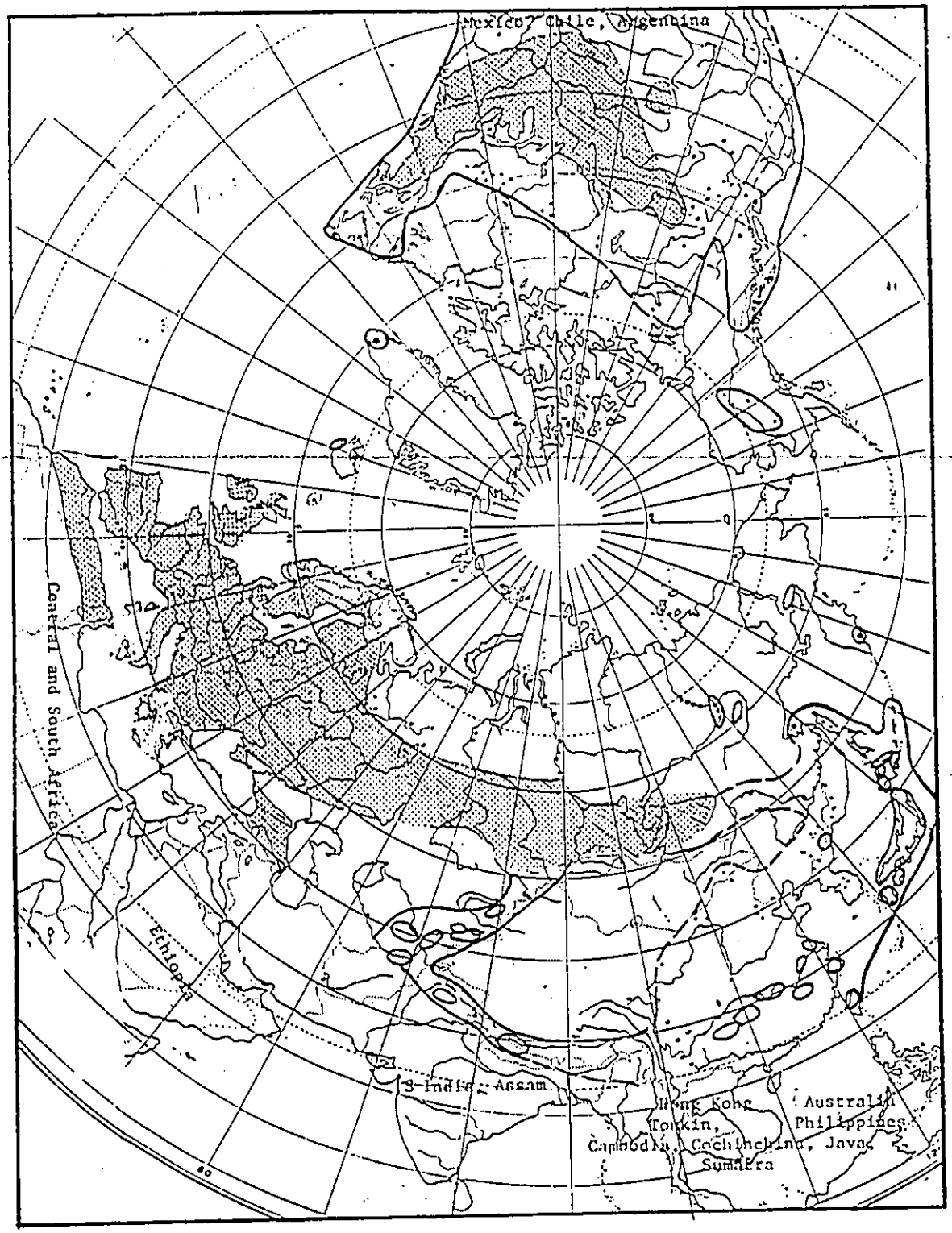

Mexico, Chile, Argentina
Central and South Africa
Ethiopia
Australia
Philippines
Hong Kong
Tonkin,
Cambodia, Cochinchina, Java,
Sumatra

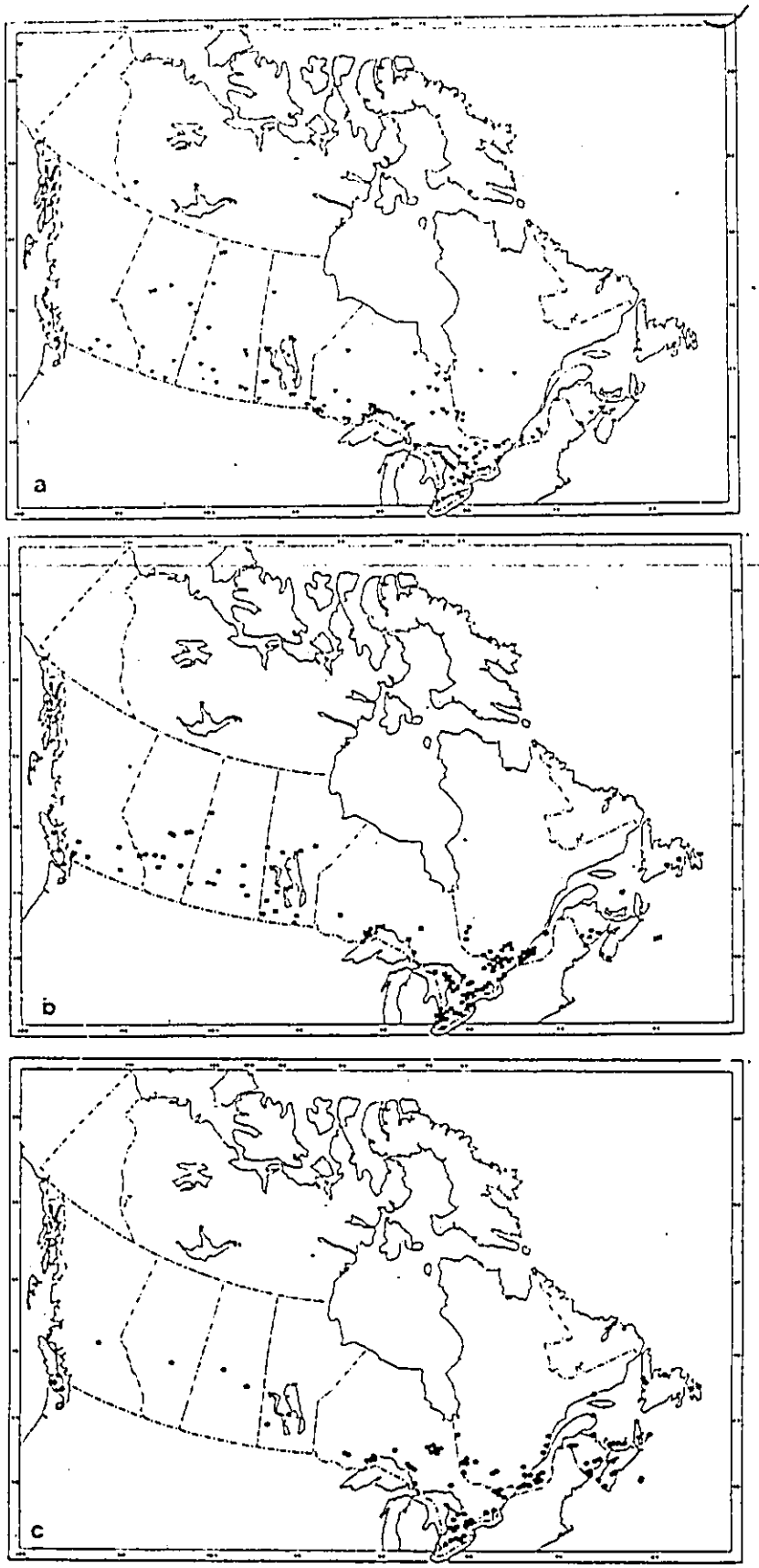
Figure 2: Distribution of Canadian specimens of:

a: P. lapathifolium var. lapathifolium,

b: P. lapathifolium var. salicifolium,

c: P. scabrum

scored from herbarium specimens at DAO, CAN, TRT, and TRTE.


a
b
c

1.3.3. Reproduction and Hybridization

Reproduction in this group of plants is primarily, but not obligately, autogamous (Knuth, 1909, Timson, 1965). There are chasmogamous flowers on every plant; authors have, however, observed that these flowers do not set fruit, but remain sterile (Hy, 1885). Rigidly controlled experiments have not been done to date that would show conclusively that such is always the fate of chasmogamous flowers in this group. Hybridization is not a common occurrence, even when several closely related species grow together. Artificial hybrids are not successful, although hybrids have been described from herbarium specimens (Timson, 1965).

1.3.4. Cytology

Different groups (sections and segregate genera) of Polygonum s.l. have characteristic chromosome base numbers (Haraldson, 1978). They are, in general, either n=10, as for example in section Polygonum, or n=11 for most of section Persicaria (Löve and Löve, 1956). Because of this difference in base numbers, Persicaria is often recognized as a separate genus.

Since inter-taxon differences in ploidy level can impose sterility barriers permitting the evolution of a distinct reproductively isolated species, the study of relative relationships among and within taxa should, therefore, begin with an investigation into their ploidy level.

Chromosome counts have previously been reported for gametophytic

TABLE 2: Chromosome counts reported from literature

Taxon	n/2n	source
Polygonum lapathifolium L. as:		
Polygonum lapathifolium	/22	Wcislo (1977)
	11/	Mulligan & Porsild (1969)
	/22	Podlech & Diertle (1969)
	/22	Jaretsky, (1927)
	/22	Tischler (1934)[1]
	/22	Pauwells (1959)[1]
	/22	Timson (1965)
	/22	Graham & Wood (1965)
	11/	Aparicio & Silvestre (1984)
	/22	Hammerton & Stone (1966)
	/22	Simmonds (1945)
Persicaria lapathifolia	/22	Löve & Löve (1948)
	/22	Andersson (Löve & Löve 1948)[1]
	/22	Löve & Löve (1956b)
	22/44	Hara (1952)[1]
P. nodosum Pers.	/22	Javurkova (1980)
	/22	Doida (1960, 1962)
	/22	Mehlinkova (1965)[1]
	/44	Löve & Löve (1942a)[R]
Persicaria lapathifolia ssp. nodosa	/22	Vachova & Zaborsky (1980)

Table 2 (cont.):

Polygonum "scabrum" Moench as:		
P. scabrum	11/	Taylor & Mulligan (1968)
	/22	Mulligan (1961)
	/44	Menshikova (1964)[1]
P. tomentosum	/22	Jaretsky (1928)
	/22	Tischler (1934)[1]
	/22	Anderson (Löve & Löve, 1942b)
	/22	Löve & Löve (1942a)
Polygonum brittingeri Opiz:	/22	Pogan & Reychlewski (1980)[1]
Polygonum persicaria L. as:		
Polygonum persicaria	/40	Weislo (1977)[R]
	22/	Taylor & Mulligan (1968)
	/22	Mehlinkova (1964)[1]
	/22,44	Queiros (1983)[R]
	/40	Uhrikova (1978)[R]
	/44	Löve & Kjellquist (1974)
	/22	Gadella & Kliphius (1966)
	/40	Doida (1960, 1962)
	/40,44	Graham & Wood (1965)[R]
	/44	Jaretzky, (1927, 1928)
	/44	Tischler (1934)[1]
	/44	Rohweder (1937)[1]
	/44	Andersson (Löve & Löve 1942b)[1]

Table 2 (cont.):

	/44	Pauwels (1959)[I]
	/44	Timson (1965)
Persicaria maculata	/44	Löve & Löve (1956b)
Polygonum pensylvanicum L. as Persicaria pensylvanica (L.) Gomez de la Maza	/22	Löve & Löve (1982)[R]

[I] = cited in Bolkhovskikh et al, 1974

[R] = requested for confirmation (see Section 2.5.1)

and sporophytic material of many taxa of Polygonum section Persicaria. A summary of those reported for the species studied here is in Table 2. It can be seen from this table that the only previously reported chromosome number for P. pensylvanicum is 2n=22, whereas those for P. scabrum, P. lapathifolium and P. nodosum were 2n=22 with one report in each case of 2n=44. Discrepancies in the number for P. persicaria are more numerous, for although most reports are of 2n=44, three of 2n=22 and four of 2n=40 exist. Therefore, it appears that P. persicaria is generally tetraploid, although the diploid ploidy level may exist within this species.

The first objective was to evaluate the literature reports of chromosome number, particularly for members of the P. lapathifolium complex. The next aim was to confirm these counts from the populations being studied, particularly where there were discrepancies in the numbers reported in the literature.

1.3.5. Morphology

A recent study on P. lapathifolium in Manitoba was performed by Roshon and Staniforth (1987) studying "autodemes". Autodemes are groups of individuals of a particular taxon that are composed of predominantly autogamous individuals (Stace, 1980). This study investigated whether slight morphological differences bred true in three of these autodemes found co-existing on a pond edge in Winnipeg. They found that most of the characters separating them in nature persisted from generation to generation. This indicates true-breeding differences in morphology that are maintained by autogamy.

As Timson (1963) described, this allows the species to exhibit considerable discontinuous intraspecific variation. Staniforth and Cavers (1979) studied the ecology of P. lapathifolium and P. scabrum. Encountering difficulties in assigning several specimens to species, they simply assigned them to the group that they had most closely resembled. These situations indicate a possible inadequacy in classification of these plants.

1.3.6. Flavonoids

Results of morphological analysis show that Polygonum persicaria, P. pensylvanicum, and the P. lapathifolium complex are generally readily distinguishable. However, in the P. lapathifolium complex morphological analysis does not always reliably resolve taxa.

Flavonoids, as a group, are relatively uniform structurally (Harborne and Turner, 1984). Of the possible flavonoid constituents, non-pigmented flavonoids were studied here. Anthocyanins were not analyzed chemically but their presence/absence was recorded under morphology. It has been noted in germination and cultivation of the specimens of Polygonum studied here that young seedlings almost always exhibit some anthocyanin production, which diminishes with development in plants of P. scabrum, and in some populations of P. lapathifolium. Anthocyanins are visible in plants of P. persicaria, P. pensylvanicum, and in most populations of the P. lapathifolium complex; the degree to which they may be found in the sepals and stems varies among, and, to a lesser extent, within populations. In previous reports of anthocyanins identified from this group, Yoshit-

ama et al. (1984) reported cyanidin-3-glucoside and peonidin-3-arabinoside-5-glucoside in the sepals of P. lapathifolium but gave no report of anthocyanins in the stem or leaf sheath. On the other hand, they found cyanidin galactoside in the stem and leaf sheath of plants they referred to as P. lapathifolium ssp. nodosum (= P. lapathifolium ssp. lapathifolium), but identified no compounds from the sepals. Cyanidin-3-glucoside and peonidin glycoside were found in the sepals of P. persicaria.

Studies on various plant groups have shown that flavonoid pattern often does not vary under different environmental conditions (McClure and Alston, 1964, 1966). In other studies (Abrahamson and Solbrig, 1970; Ball et al., 1967), some environmental influences were found to affect chromatographic results; stage of development and organs analyzed also affected, to some extent, the flavonoid pattern observed (Parks et al., 1972; Classen and Nozzolillo, 1981). Therefore, for preliminary studies of the flavonoid content in the species studied here, analysis was performed on leaf and stem material of young, non-flowering plants, which as far as possible were grown under identical conditions. Isobe et al. (1979, 1980) have reported quercetin-3-β-D-glucoside-2"-gallate, quercetin, kaempferol, quercetin-3-O-glucoside, and kaempferol-3-0-β-D-glucopyranoside-2"-gallate from P. nodosum in Japan, using both leaf and stem material.

The patterns of non-pigmented flavonoid compounds were studied in the four North American taxa, emphasizing in particular the relationships among the taxa of the P. lapathifolium complex.

1.3.7. Electrophoretic evidence

1.3.7.1. Advantages of the Electrophoretic technique

In systematic and phylogenetic studies, morphological attributes and even secondary metabolites such as flavonoids have often been inadequate representations of genotype for the resolution of the taxonomy of closely related groups of plants. This is because of the difficulty in equating phenotype with genotype (Gottlieb 1971, 1977) since:

a) the majority of morphological features in plants are controlled by more than one gene, and alleles at each locus contribute to these attributes;

b) many morphological features are environmentally influenced;

c) the synthesis of secondary compounds is controlled by gene regulation at various steps along biosynthetic pathways.

In electrophoretic evidence, the equation between phenotype and genotype is more clearly understood (Gottlieb, 1977). Isozymes are different forms of an enzyme coded for at different gene loci. Allozymes are different forms of the enzyme coded for at a single polymorphic locus. A taxonomist uses electrophoretic data by investigating the amount of genetic variation represented by multiple alleles in the pattern of isozymes which occur in a group under study. The mobility of enzyme bands in an electrophoretic gel is related to the charge of the polypeptides, and to some extent, their size, which are a consequence of their amino acid sequences. Since these sequences are colinear with the nucleotide sequences coding for

them, "an analysis of a protein structure using electrophoresis is, to a first approximation, an analysis of a gene" (Gottlieb, 1977).

Gottlieb (1977) and Crawford (1983) summarize the advantages of electrophoretic evidence in plant systematics. Electrophoresis allows comparisons between gene products that are homologous. This avoids problems such as convergence that often arise when studying morphological characters. In addition, the amount of genetic information available can be quantified directly and stated exactly, which is often not possible with other data sources. The problems of absence of characters that may arise with secondary compounds or morphological analysis are generally avoided with this technique because if one form of an enzyme is absent, an alternative one will usually be present.

Several limitations to this technique must be recognized, for example:

a) only 30% of nucleotide changes result in changes in electrophoretic mobility;

b) generally, only 15-25 enzymes are studied. The data set is, therefore, limited;

c) from the differences in electrophoretic mobility, one cannot determine the exact number of nucleotide substitutions involved.

Even though limitations exist, the direction of bias is known. All in all, electrophoretic evidence provides an underestimate of the actual genetic variation between taxa, and this makes it a strong tool for studying phenotypic variation in plants. In addition, the enzymes chosen are those involved with essential biosynthetic

pathways. They are, therefore, some of the least likely characters to be under selection pressure from environmental influences.

1.3.7.2. Taxonomic problem solving using electrophoresis

Electrophoresis has been very helpful in sorting out complicated variation patterns within plant groups at the species level and below (see Gottlieb, 1977 and Crawford, 1983 for examples). One such case is the Carex crinita complex in which taxonomic treatments previous to the work of Bruederle and Fairbrothers (1986) had differed considerably, with three to seven taxa of varying rank being recognized. The study of allozyme variation in the complex resulted in the recognition of four species. Warwick and Aiken (1986) provided electrophoretic evidence that two species of wild-rice (Zizania aquatica and Z. palustris) should be recognized, instead of only one as treated by Fassett (1924) and several subsequent authors. This evidence supported conclusions from previous morphological studies (Dore, 1969; Dore & McNeill, 1980). Thus, when a problem of species recognition within a group of plants exists, electrophoretic evidence can often provide an additional source of genetic information that to test proposed classifications and species relationships.

In some cases allozyme evidence is incongruent with findings from morphological and other data sources. Such cases have been described, for example, by Warwick (1987) for proso millet, Gottlieb (1973b, 1978a,b) for Stephanomeria, Gottlieb and Pilz (1976) for Gaura longiflora and Soltis (1982) in Sullivantia, to name a few examples.

It is important not to rely solely on electrophoretic evidence for taxonomic conclusions, since it has been shown that, ultimately, there is no necessary correlation between variability of allozymes and cytological, morphological and chemical variability. With gradual speciation showing divergence over a period of time, one would expect a correlation of these data sets; however, under rapid or abrupt speciation, or recent divergence, there may be no correlation (Crawford, 1985).

1.3.7.3. Enzymes and Polyploidy

Evolutionary histories of polyploid taxa can be partially determined by the electrophoretic technique. First, a polyploid can be recognized by the presence of extra isozymes that reveals a duplication of the genome (Gottlieb, 1977). Secondly, it is possible to distinguish whether a taxon has arisen from autogamous polyploidy or is an alloploid. The study of the tetraploids Tragopogon mirus and T. miscellus and their diploid progenitors, by Roose and Gottlieb (1976), showed that the tetraploids possess enzymes contributed by both parents, plus novel enzymes developed after the speciation event. In autopolyploid taxa, almost no alleles are found that are not found in the parent. This situation was found by Crawford and Smith (1984) for the hexaploid Coreopsis grandiflora var. longipes, and by Epes and Soltis (1984) in Galax urceolata. There should also be evidence of gene dosage effects in the autogamous polyploid, as found at the Per-2 locus in Atriplex canescens, where diploids stained lightly, tetraploids darker and hexaploids darkest (McArthur

et al., 1986). Soltis and Reisenberg (1986) emphasize the fact that evidence from diverse sources must be examined before a true autopolyploid can be documented. Their electrophoretic study of Tolmiea menziesii afforded supporting evidence for the autopolyploid origin proposed for it in an earlier study (Soltis, 1984), as well as providing insights into autopolyploid speciation.

1.4. Aim of this study

Earlier studies using morphology alone have not been completely successful in resolving the classification of taxa of the P. lapathifolium complex in the Northern Hemisphere. The relationships of P. pensylvanicum and P. persicaria to these taxa also bears investigation.

The overall hypothesis of this study is that present treatments in North American floras are not adequate to represent the variation found in the P. lapathifolium complex.

Several sources of taxonomic information: cytology, flavonoid chemistry, morphology and electrophoretic evidence will be employed. The emphasis will be placed upon comparing electrophoretic data with the other three sources of information. It is hoped that, in the study of this group, isozyme variation patterns will reveal relationships among these taxa, and answer the following questions:

i. Is the variation within the P. lapathifolium complex a subset of the extensive variation in Europe, or are there genetically recognizable North American taxa?

ii. At what taxonomic level should variation exhibited be recognized, e.g., does P. scabrum warrant species status?

iii. Do electrophoretic patterns support previous reports of ploidy level?

iv. Do P. persicaria and P. pensylvanicum show allopolyploid or autopolyploid derivation, and from which taxa did they arise?

2. MATERIALS AND METHODS

2.1. Herbarium material

North American material from DAO, TRT, TRTE and CAN (acronyms as defined by Holmgren and Keuken, 1981) recognizable as P. lapathifolium var. lapathifolium, P. lapathifolium var. salicifolium and P. scabrum (560 specimens) was examined. Characters which varied within species were noted, and the percentage of specimens that could not be identified by key characteristics in Fernald (1950) or Scoggan (1978) was determined. European specimens of plants in the P. lapathifolium complex (140) from DAO and CAN were compared with those from North America. Data on the lectotype[1] of P. lapathifolium L. (Hort. Cliff. 42, Tourn. Inst. 510: BM) selected by Timson (1963) was provided by J. McNeill on the basis of a character questionnaire that I prepared (questionnaire explained in Appendix C).

2.2. Site choice and Study

Sites in Ontario and Quebec obtained from herbarium specimens (see above) were visited from July to October, 1985. Other collections were made in Colorado (J. McNeill), in the Ottawa region, southern Ontario, and northern New York in 1984 and 1985. The

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[1] Lectotype: "is a specimen or other element selected from the original material to serve as a nomenclatural type" (element to which the name of a taxon is attached) "when no holotype was indicated at the time of publication or as long as it is missing." (Stafleu, 1983).

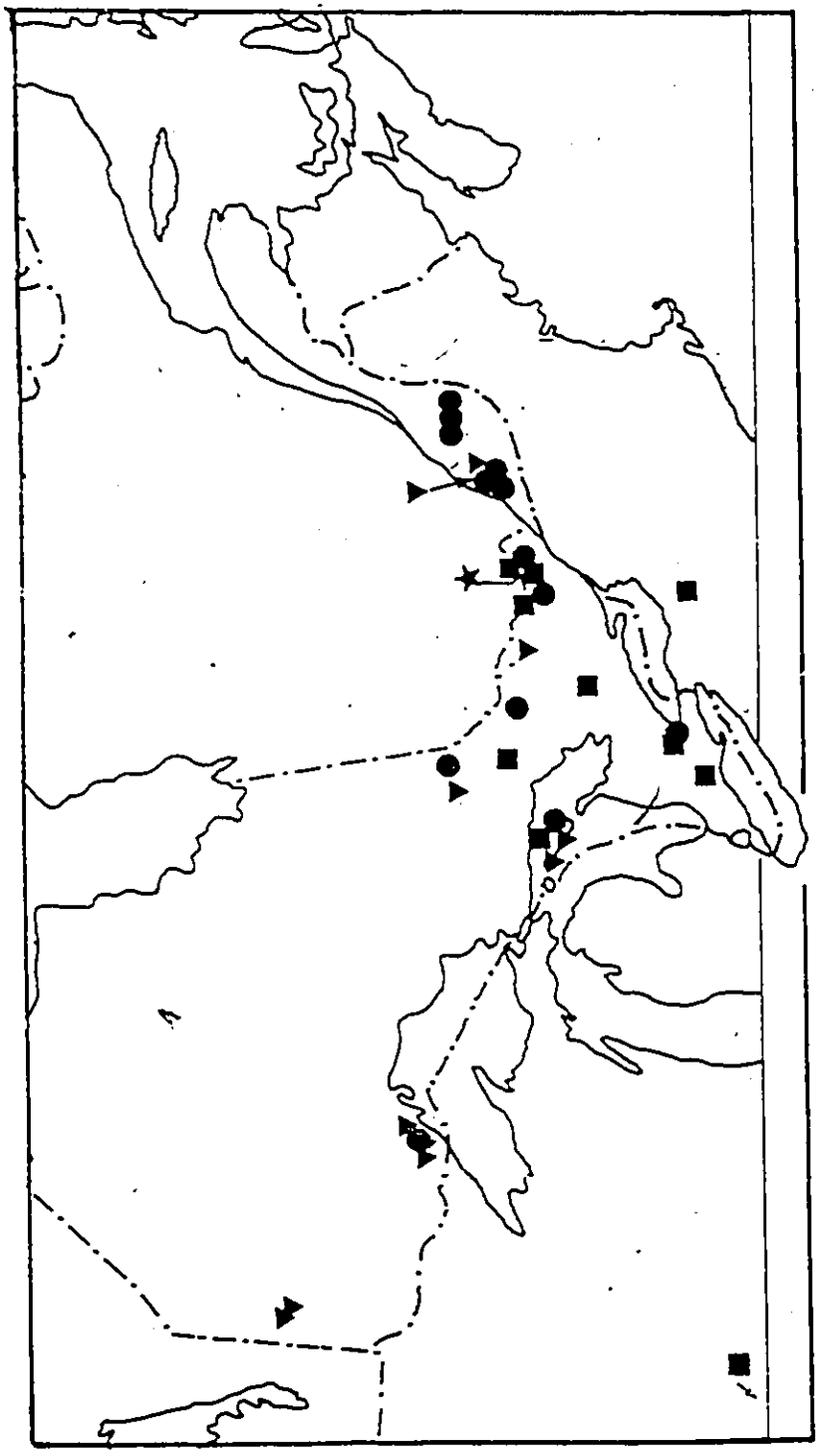
collection sites are shown in Figure 3. The site descriptions are given in Appendix A. A mixed population of P. lapathifolium and P. scabrum, visited in 1984, at the St. Isidore de Prescott sewage lagoons (* in Figure 3) was surveyed in more detail in 1985. Fifty plants of P. lapathifolium var. lapathifolium, 50 of P. scabrum, and 50 intermediate plants were collected in 1985 at this site.

Seventeen seed accessions were obtained from 11 botanical gardens in Europe. These were sent to me under the names P. lapathifolium (7) (2 to be referred to as P. lapathifolium ssp. lapathifolium; 5 as P. lapathifolium ssp. tomentosum), P. lapathifolium ssp. lapathifolium (3) (=P. lapathifolium ssp. tomentosum), P. lapathifolium ssp. nodosum (Pers.) Danser (2) (to be referred to as P. lapathifolium ssp. lapathifolium), and P. persicaria L. (5) One seed collection was obtained from T. Isobe in Japan, under the name P. nodosum (= P. lapathifolium ssp. lapathifolium). The above accessions were all collected in 1984 and 1985 from natural localities. The variation found within these 'populations' will be analyzed recognizing that the exact number of parent plants is not known. In addition, plants (with seed) of three populations of P. lapathifolium L. ssp. tomentosum and three of P. persicaria were collected by J. McNeill in Austria and England in 1985.

2.3 Germination and growth of seedlings.

Achenes were germinated in the laboratory at the University of Ottawa. They were washed for 30 seconds in 1.5% sodium hypochlorite,

Figure 3: Collection sites of P. lapathifolium and P. scabrum, 1984 and 1985.
Circles=P. scabrum
Squares=P. lapathifolium var. lapathifolium
Triangles=P. lapathifolium var. salicifolium.
Descriptions of collection sites in Appendix A.



rinsed 3 times for 30 seconds each in double distilled water and then either germinated immediately or cold treated (stored in double distilled water at 5.5°C for a month) and then germinated. Placed between three layers of Watman #1 filter paper (1 on top and 2 on bottom) in a sterile petri dish they were kept moist with double distilled water under a 10 hour light regime at 3874 lux and 21°C until the radicle appeared, usually within one week.

Percent germination was recorded and some of the ungerminated seed tested with 0.1% 2,3,5-tetrazolium chloride to determine the level of viability (Delouche et al., 1962).

After 1 1/2 weeks, seedlings were transferred to 7.5cm X 5.0cm pots in a 2:1:1 soil:sand:peat mixture, and grown under alternating temperature conditions (25°C day, 21°C night) with supplementary bench lighting for a 16 hour day (1.8 X 10^4lx), fertilized every two weeks and scored for subsequent measurements. As far as possible, plants were grown to seed-set and then harvested as vouchers to be deposited at the Vascular Plant Herbarium, Agriculture Canada, Ottawa.

2.4. Flowering

The number of weeks to flowering was recorded for all plants of the P. lapathifolium complex when grown (a) at the Central Experimental Farm in Ottawa under conditions described above, and (b) the University of Ottawa greenhouse under the same conditions but fertilized only once a month. The means and standard deviations of the time to flowering were calculated for each population.

2.5. Cytology

2.5.1. Evaluation of Previous Chromosome Counts

Specimens from the institutions marked with "R" in Table 2 were requested on loan (only one was received) to confirm the identity of the vouchers from which counts of 2n=44 were reported for P. lapathifolium, P. scabrum and P. nodosum, 2n=22 for P. pensylvanicum, and 2n=22 and 2n=40 for P. persicaria.

2.5.2. Experimental

Cytological analysis was performed on gametophytic material from young buds of North American populations of P. lapathifolium var. lapathifolium, P. lapathifolium var. salicifolium, P. persicaria, P. scabrum, and P. pensylvanicum.

Buds were collected at first signs of flowering, that is, when the apical new leaves showed a "bulge" indicating the presence of an incipient inflorescence. They were immersed in a fixative of fresh 10:3 95% ethanol:acetic acid (Darlington and LaCour, 1962), and fixed for 3 to 8 hours, after which the inflorescences were washed three times in 70% ethanol and subsequently stored for up to 2 months at a temperature of -7°C. The buds were stained in aceto-orcein (prepared following the method described in Mertens, 1969).

2.6. Morphology

2.6.1. Data sets

The analyses were performed on three main datasets (Table 3).

Table 3: Data Sets Used in the morphological analysis. Population codes given in Appendix A.

Data Set #	Total # Individuals	Taxa
1 Field collections, plants of all taxa.	153	P. lapathifolium: LA1, LA2, LA5, LA6 P. pensylvanicum: NP1, NP2, NP3, NP4, NP5 P. persicaria: PS1, PS2, PS3, PS4, PS5, PS6 P. scabrum: SC1, SC2, SC3, SC4, SC5, SC6 Numbers given in Appendix B.
2 Field collections and cultivated plants of the P. lapathifolium complex.	307	P. lapathifolium var. lapathifolium, P. lapathifolium var. salicifolium, P. scabrum, (European) P. lapathifolium ssp. lapathifolium, (European) P. lapathifolium ssp. tomentosum, Numbers given in Appendix B.
3 Field collections, plants of the P. lapathifolium complex from mixed population.	89	P. lapathifolium var. lapathifolium (30), P. scabrum (30), Intermediates (29). All from population SL1.

The first (1) contained data from field collections of all taxa (one fruit analyzed per plant); the second (2) contained all data of field and cultivated plants of the P. lapathifolium complex (3 fruits per plant); Plants grown to maturity in greenhouse conditions ("cultivated") were included to investigate the possible interference of phenotypic plasticity in the statistical analysis among the putative taxa. The third (3) contained the data from 89 field collected plants from the water treatment pond population (SL1) (list of OTUs given in Appendix B). This final dataset was included because this population contained P. lapathifolium, P. scabrum and plants identified as intermediates, thus the former two taxa could be investigated as possible parents of hybrid individuals.

2.6.2. Characters analyzed

The characters measured are described in Table 4 and illustrated in Figure 4. The measurements of perianth, achene and cilia length were done on a Zeiss binocular dissecting microscope, with the scale units 1=0.0625mm (1=0.0125mm for cilia length). The more accurate higher magnification was not used on the perianth or achene because many of the fruits were too large to fit on the scale. The counts of number of glands are often approximate (+/- 10%), as it was sometimes difficult to determine whether several glands were merged together.

For dataset #1, characters 1 to 10 were analyzed; for datasets #2 and #3, characters 1, 2, 4-16 were analyzed univariately and characters 1, 2, 4-10 and 13-16 were included in the principal components analysis.

Table 4: Characters scored in morphological analysis for all data sets.

Fruit characters (Figure 4: a, b, c)

1. Length of perianth (micrometer units = 0.0625 mm)
2. Width of perianth (micrometer units = 0.0625 mm)
3. Presence/absence of recurved veins on perianth
4. Number of glands on entire perianth. If over 100, and/or merged together, numbers approximate to 10-15%
5. Length of achene (micrometer units = 0.0625 mm)
6. Width of achene at widest point (micrometer units = 0.0625 mm)
7. Distance from base of achene to widest point
8. Intensity of chlorophyll in perianth (0-3)
   (0=none, 1=slight green, 2=deeper green, 3=intense green)*
9. Intensity of pink anthocyanin in perianth (0-3)
   (0=none, 1=slight pink, 2=deeper pink, 3=almost red)*
10. Position of pink in perianth (0-3 or no data)
    (0=none, 1=tip, 2=along an edge, 3=all over)*

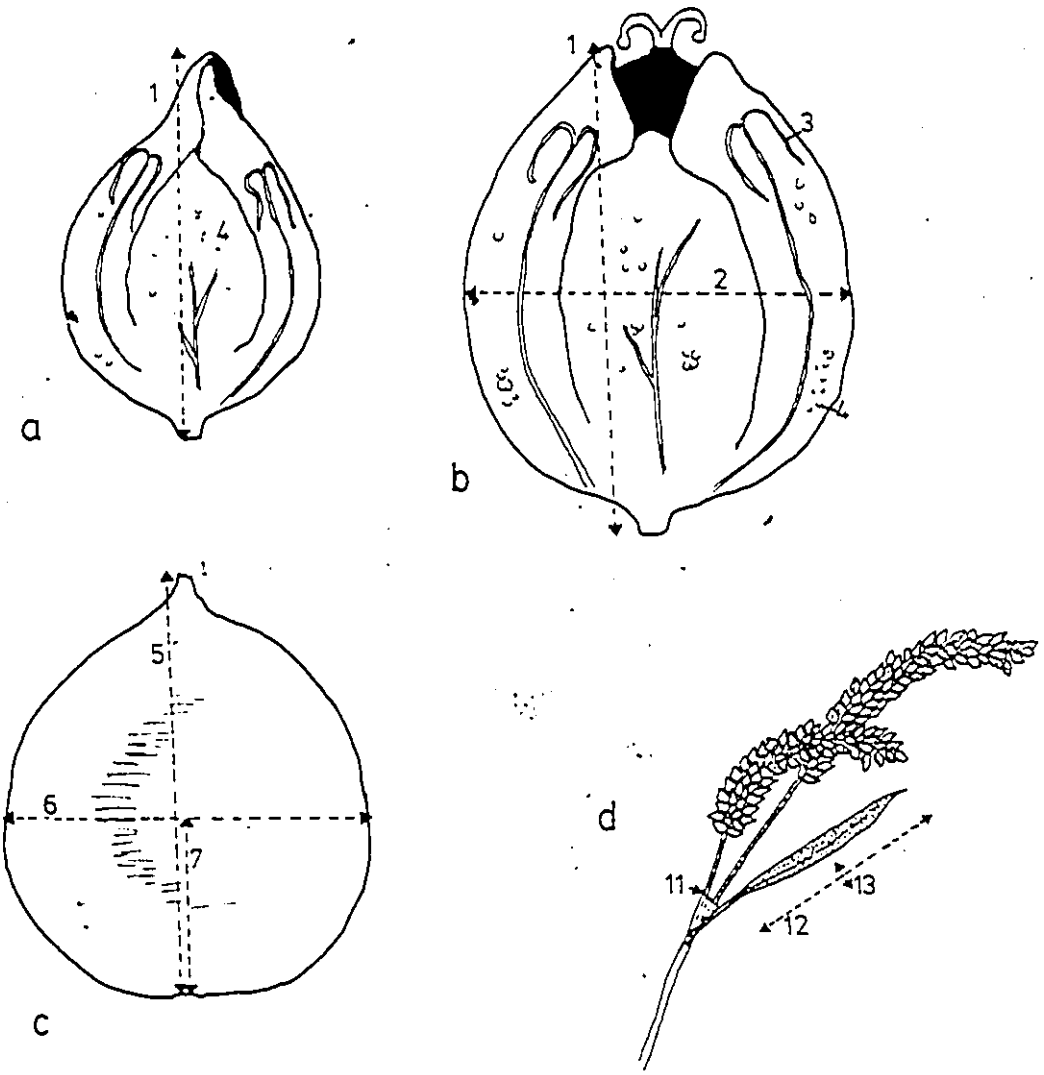
Vegetative characters (Figure 4d)

11. Length of cilia on first ochrea below main inflorescence (micrometer units = 0.125 mm)
12. Length of 1st leaf below main inflorescence (mm)
13. Width of 1st leaf below main inflorescence (mm)
14. Length of 4th leaf below main inflorescence (mm)
15. Width of 4th leaf below main inflorescence (mm)
16. Height of flowering plant (cm)

*for characters 8, 9 & 10 a colour chart was made and used for all measurments

Figure 4: Characters measured in morphological analyses. a: typical P. lapathifolium fruit, b: typical P. scabrum fruit c: achene d: terminal inflorescence.

1. Length of perianth
2. Width of perianth at widest point
3. Presence/absence of recurved veins on perianth
4. Number of glands on perianth
5. Length of achene
6. Width of achene at widest point
7. Distance from base of achene to widest point
11. Length of cilia on ochreae
12. Length of first leaf below main inflorescence
13. Width of first leaf below main inflorescence.


1
4
a
1
3
2
b
5
6
7
c
d
11
13
12

2.6.3. Transformation of data and Univariate analysis

The character measurements of datasets 1 and 2 were plotted by a SAS program (Ray, 1982) to determine the pairwise relationships between the characters for the individuals. Charts were constructed for each character individually by a SAS program and the Agriculture Canada Engineering and Statistical Research S199 program (Wolynetz and Thompson, 1977) to check the distribution for skewed data. Square root and logarithmic transformations were tested to determine whether these transformations would reduce departures from normality in the characters. The character "number of glands" was transformed by taking the square root, the logical transformation as the character is a function of area (Sneath and Sokal, 1973). This guarded against a few very large values dominating the analysis inappropriately, as can happen with a highly skewed distribution. The appropriateness of the transformation was confirmed by the fact that the square root of the number of glands was found to have a distribution closer to the normal curve than that of the untransformed data. The other characters were not transformed (although residuals were calculated for some characters as described below), because no transformation improved the normality of the distribution of any to a degree sufficient to justify transformation.

A size component particularly evident in the length of the perianth greatly influenced the analyses of these taxa, and character #1, length of perianth, did not significantly separate the taxa. Thus, regressions were performed on the length of the perianth to the

other size characters of the fruit. The residual values of these regressions for the latter three characters were then tested in subsequent principal components analyses. The effect of taxonomically unimportant size was thus reduced, clarifying the relationships among the taxa. Residuals were, therefore, were used in the remainder of the analyses.

A one-way analysis of variance was performed on each of the characters, using the general linear model of the SAS statistical package because of slightly unequal sample sizes (Ray, 1982, p. 119). This was performed to see whether the individual groups could statistically have come from the same population. The four originally assigned species were used as groups in the first dataset (4 groups). The seven groups in the second dataset consisted of six groups corresponding to wild and cultivated P. lapathifolium var. lapathifolium, P. lapathifolium var. salicifolium, and P. scabrum, respectively; plus a seventh group consisting of cultivated European plants (ssp. lapathifolium and ssp. tomentosum). The latter group was included as a comparison with American plants. A nested analysis of variance was performed on each character in the second data set testing for significant differences among the seven groups, among populations within taxa (73 groups), and among plants within populations for each taxon (307 individuals). To determine where the significant variation lay, pairwise multiple comparisons were performed on the means of the taxa and populations, using the Tukey-Kramer option of Duncan's multiple range test because of unequal population sizes.

2.6.4. Multivariate analyses

Cluster analyses encompass a group of techniques that define clusters of mutually similar entities from resemblance matrices. Clustering was performed here under the CLUSTRIT procedure (Lefkovitch, 1981). Average linkage, single linkage and flexible sorting algorithms were employed, the last with alpha = 0.625. In these analyses the Gower coefficient (Gower, 1971) was used, in which the data are standardized by range; this allows the use of mixed data (combination of continuous, discontinuous and qualitative data) by specifying the type of each variable, and results in a Manhattan distance by conversion to dissimilarity (Lefkovitch, 1981; Sneath & Sokal, 1973). The results were drawn in the form of a dendrogram, depicting the clustering relationships. In clustering techniques, groups will be formed regardless of whether or not the resulting structure is meaningful in nature.

Principal components analysis (PCA) seeks to partition the information contained in several variables into fewer, synthetic, uncorrelated variables, or components. The procedure uses eigenanalysis to extract these components. The component scores were plotted to represent the spacial relationships of the individuals on the component axes. The percentage of total variance represented on each component axis and the percentage of total variance per descriptor accounted for by each component was calculated to aid in interpretation. This multivariate ordination procedure (PRINCOMP procedure in SAS) was used to perform this analysis.

2.7 Flavonoid Analysis

Plants were grown from seed in the University of Ottawa greenhouse for six weeks: by which time at least five leaves had opened. Specimens analyzed are indicated in Appendix B. Leaves were dried overnight at ca. 37°C and stored in the dark, at room temperature and kept wrapped in plastic (protected from moisture) for subsequent analysis. Various pre-analysis storage treatments did not produce any variation in the chromatographic patterns of these stable compounds.

The flavonoids were extracted from finely chopped leaf material by the method described by Classen and Nozzolillo (1980), originally developed by F. W. Collins, Agriculture Canada, Ottawa.

A 75 X 75 mm sheet of Polyamide microlayer (ML 1515, Mandel Scientific Co.) was spotted with 0.005ml of extract and run in the aqueous solvent system (water: pyridine: cyclohexanone, 90:5:5), dried, rotated through 90° and run in the organic solvent (n-butyl acetate: methanol: formic acid: water, 60:30:5:5). The plates were then sprayed with 1% diphenylboric acid ethanolamine complex (flavone reagent) in water containing 1% triethylamine; then observed under long wave uv light. Compounds were tentatively identified as quercetin, kaempferol and isorhamnetin glycosides on basis of colour before and after spraying, viewed under uv light. Levels of glycosylation were estimated by degree of mobility in the aqueous and organic solvents.

The flavonoids were each designated by a number, and presence and absence of each compound was recorded for all specimens.

The standards used were: naringin, rutin, chlorogenic acid, quercetin-3-rhamnose, Kaempferol, gallic acid (C. Nozzolillo, University of Ottawa); quercetin-3-O-glucoside-2"-gallate, kaempferol-3-O-glucoside-2"-gallate, myricetin-3-O-rhamnoside, quercetin-3-O-rhamnoside-2"-gallate (T. Isobe, Dept. of Chemistry, Hyogo, Japan).

2.8 Electrophoresis

Electrophoretic surveys were performed on one to four seedlings grown from seed of one to ten plants from each of the 72 North American, European and Japanese populations (Appendix B). Voucher specimens for the collections analyzed are deposited at the Vascular Plant Herbarium, Agriculture Canada, Ottawa. The number of plants analyzed is summarized in Table 5.

The surveys were performed on seedlings that were 3 to 5 weeks old, using approximately 70 mg of tissue from the youngest leaves. The samples were ground in small plastic weighing boats using approximately 60 uL of extraction buffer as in Gottlieb (1981c), with about 12 mg of polyvinylpolypyrrolidine to bind phenolics. Exact quantities of buffer and plant material were not used (except where later described for quantitative analyses of two enzyme systems). Electrophoresis was conducted on 9.5% starch gels using different electrode and gel buffers depending on which of the 15 enzymes was to be analyzed. The buffer systems used were similar to those of

Table 5: Number of Progeny and Parental Plants analyzed electrophoretically (refer to Appendix B for numbers of parental plants analyzed per population).

TAXON	Total # of progeny/ total # parental plants
P. pensylvanicum	33/16
P. persicaria	98/46
P. lapathifolium var. lapathifolium	133/37
P. lapathifolium var. salicifolium	52/28
P. scabrum	80/53
mixed population (P. lapathifolium, both varieties, & P. scabrum)	141/58
European (P. lapathifolium s.l.)	58/52

Gottlieb (1981c), Warwick and Gottlieb (1985) and Warwick and Black (1986). Staining for enzymes followed standard procedures of Gottlieb (1981c) and Warwick and Gottlieb (1986) (see Appendix D). A tris-citric acid system at pH 8.3 (Gottlieb, 1981c) was used for glutamate dehydrogenase (GDH), glyceraldehyde-3 phosphate dehydrogenase (G3PD), leucine aminopeptidase (LAP), triose phosphate isomerase (TPI), and phosphoglucose isomerase (PGI). A tris-citric acid system at pH 8.8 (Gottlieb, 1981c) was employed for hexokinase (HK) and glutamate-oxaloacetate transaminase (GOT). A histidine/HCl system at pH 7.0 (Warwick and Black, 1986) at 20 mM or 15 mM was used for mannose phosphate isomerase (MPI) and phosphoglucose mutase (PGM), and at 15 mM or 10 mM for shikimic acid dehydrogenase (SKDH) and 6-phosphogluconate dehydrogenase (6PGD). A histidine system at pH 5.7 (Warwick and Black, 1986) was used for malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH) and aldolase (ALDO). The isozymes migrated toward the anode in all of these systems.

Although it was frequently difficult to determine the genetic bases of the electrophoretic patterns because of the lack of variation among individuals for most isozyme patterns plus the presence of several fixed multi-banded patterns, loci were tentatively assigned for each enzyme, except TPI for the tetraploid species, which had 5- and 6-banded patterns that were difficult to interpret. The most anodal locus was called 1, the next 2. At each locus, the alleles specifying the allozymes were described by their mobilities relative to the bromphenol blue front for all systems.

Initial surveying of isozymes revealed distinct differences for

several enzyme system patterns among (a) P. persicaria, (b) P. pensylvanicum and (c) the P. lapathifolium complex. Therefore, the subsequent surveys focussed on the patterns within the P. lapathifolium complex. In addition, because of the very low variation within and between populations of the P. lapathifolium complex in initial runs of the 1984 collections, a small sample of a more geographically widespread set of populations was collected and surveyed in 1985.

Putative heterozygous individuals at two enzyme loci were selfed and segregation ratios were examined in the progeny to determine the genetic bases for the banding patterns.

Three populations, L18, L12, and L36, had slightly different patterns of TPI and ACON. For each enzyme, one of the allozyme bands was consistently less darkly stained in these populations. Therefore surveys were performed using known quantities of material (70mg tissue, 60ml extraction buffer and 12mg PVPP) for several populations of P. lapathifolium, in order to compare the pattern in population L18 with those individuals showing the more common banding pattern.

3. RESULTS

3.1. Herbarium material

3.1.1. Identification of herbarium specimens

North American specimens

Plants of P. lapathifolium L. var. lapathifolium and P. scabrum from North America were able to be identified quite easily, using key characteristics described by Fernald (1950). The plants which appeared to key out to P. lapathifolium var. salicifolium were the most problem-posing specimens of the complex by the frequent possession of features supposedly characteristic of var. lapathifolium or P. scabrum.

A character not described in North American Floras except with respect to P. persicaria but which was noted upon examination of specimens was the varying degree to which the ochreae were ciliate. In var. lapathifolium these cilia are generally lacking, or very short, with or without glandular tips. Var. salicifolium often possesses longer cilia, and in P. scabrum, these cilia are generally visible to the naked eye (1-1.5 mm long) and lack glandular tips, giving the ochreae a scabrous edge. This character, in combination with glandularity of the peduncle was of great use in distinguishing P. scabrum from P. lapathifolium. Often, these characters were not found in these combinations; i.e. one saw profuse glandularity with few, short cilia remaining or long cilia with sparse, small glands.

Examples of these latter character combinations in freshly pressed cultivated specimens are shown in Figure 5.

About 5% of the herbarium specimens could not be clearly identified as P. lapathifolium or P. scabrum, and 15% of P. lapathifolium specimens could not be assigned to variety.

b) European specimens

These were much more variable than the North American specimens and more often combined features that were characteristic of the North American taxa. Some of the specimens could be accomodated in the three North American taxa, while others could not. The European name for intermediate plants, ssp. mesomorphum (see Section 1.3.1.1.), is often applicable (Table 6).

3.1.2. Site study

Habitat:

All of the taxa were found in a diversity of open habitats; there were, however, some tendencies for each of the three diploid taxa to occur more commonly in particular situations.

Polygonum scabrum was found in ditches, but more commonly in gravelly or dry fields, pathways or old roads, and sand or gravel bars. Polygonum lapathifolium var. lapathifolium was found most commonly in more fertile areas: beds of receded ponds or rivers, ditches and mud areas. Polygonum lapathifolium var. salicifolium was found generally on sandy shores of lakes or ponds, in areas, for the most part, less disturbed by man. Populations of this taxon were

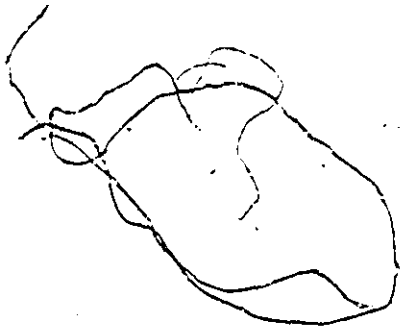


Figure 5: Photographs illustrating examples of the range of degree of glandularity of peduncles and length of cilia on ochreae found in North American populations of the P. lapathifolium complex.

a: P. scabrum from SC1

b: P. lapathifolium from LA3

Population codes: see Appendix A.

Table 6: Examples of character combinations observed on examination of herbarium specimens from CAN and DAO in 1984

Exemplar Specimen and Character combination	Name that would accomodate specimen in North America	Name according to Hegi (1931)
DAO No. 640043 as P. nodosum - glands on undersides of leaves, pinkish infl., red in stem. [Turkey]	P. lapathifolium var. lapathifolium	P. lapathifolium ssp. lapathifolium
DAO No. 651290 as P. lapathifolium ssp. pallidum -short inflorescence, pale plant, few glands. [Finland]	P. lapathifolium var. salicifolium	P. lapathifolium ssp. lapathifolium
DAO No. 279390 as ? glandular peduncle, tomentum on underside of some leaves, fat greenish inflorescence [Helsinki, Finland]	P. scabrum	P. lapathifolium ssp. pallidum (= ssp. tomentosum)
DAO No. 15523 as P. lapathifolium ssp. pallidum red spots on stem, bright pink inflorescence, narrow pale leaves with tomentum on undersides [Sweden]	?	P. lapathifolium ssp. lapathifolium
CAN No. 471513 as ? no cilia or scabrosity on ochreae, pink inflorescence, glands on peduncle, tomentous leaves achene enclosed in calyx [Sweden]	?	P. lapathifolium ssp. mesomorphum
CAN No. 129649 as P. lapathifolium quite glandular all over inflorescence, achenes enclosed in calyx, some cilia on ochreae, bottom leaves tomentous [Sweden]	?	P. lapathifolium ssp. mesomorphum
CAN No. 129652 as P. lapathifolium Achenes >2.5mm, exserted from calyx, coloured inflorescence no cilia on ochreae, no glands, some tomentum on leaves [Denmark]	?	P. lapathifolium ssp. pallidum (=ssp. tomentosum)

generally very small, in some cases consisting of only 1 to 3 plants. Exceptions to these general habitat preferences are exemplified by population SC9 (P. scabrum), growing along a northern river, and population F35 (P. lapathifolium var. salicifolium) growing along shale-covered gravel on a road shoulder along the side of a bridge.

Mixed populations composed of all three of these taxa plus P. persicaria and P. pensylvanicum were found in mud along shores of water treatment ponds, and along polluted shorelines (Fig. 6).

3.2. Germination

All populations of P. scabrum had at least 6% and an average of 56% germination without any stratification or after-ripening (Appendix D). No seeds from North American populations that were composed only of P. lapathifolium of either variety germinated without a cold treatment (5.5°C) of at least one month. After stratification, 35% germination was attained for P. lapathifolium. This was not significantly different from the germination of P. scabrum after stratification ($p>0.01$). In the mixed population (dataset #3) specimens identified as var. lapathifolium and a few of those identified as intermediate plants germinated without stratification (50.3% and 29% germination, respectively). A small percentage of the seed from the mixed population that did not germinate showed viability with the 2,3,5-tetrazolium chloride test (see Appendix D).

Seed of the populations of P. pensylvanicum and P. persicaria examined in this study required stratification to break dormancy and germinate. Germination of P. pensylvanicum was overall extremely

Figure 6: A typical habitat for the P. lapathifolium complex: P. lapathifolium var. lapathifolium at Dundas Marsh, Hamilton, Ontario.

low: 14%. Polygonum persicaria had a mean germination of 44% after 1 month of stratification.

When size of seed was examined against percentage germination after stratification (Figure 7), no statistical correlation was found. Figure 7 reveals, however, that there is a tendency for P. scabrum and European P. l. ssp. tomentosum to have larger achenes and higher germination percentage than P. lapathifolium and European ssp. lapathifolium.

3.3. Flowering

Figure 8 shows the ranges of times to first flowering, in weeks, for the diploid populations. It can be seen that most of the P. scabrum specimens germinated before 10 weeks, as did the majority of European P. lapathifolium ssp. tomentosum. Most of the North American P. lapathifolium and European ssp. nodosum flowered after 10 weeks. However, much overlap is seen between the North American taxa P. scabrum and P. lapathifolium. The means for North American P. lapathifolium and P. scabrum are significantly different. ($F = 10.29$, $p<0.01$).

When weeks to flowering were plotted against percentage germination after stratification (Figure 9), a significant negative correlation was found ($r=-0.63$ for data set #2; $r=-0.60$ for data set #3, $p<0.001$). It is seen that the majority of this variation is due to the different responses by the different initially identified taxa. Supporting this, in the mixed population, all plants identified as P. scabrum (SM in Fig. 8) had flowered by 9 weeks (mean = 5.8, 1.6 st.

Figure 7: Plot of percentage germination after stratification vs. size of achene (as length X width).

S= P. scabrum (N. American)

L= P. lapathifolium var. lapathifolium (N. Amer.)

F= P. lapathifolium var. salicifolium (N. Amer.)

E= P. lapathifolium ssp. tomentosum (European)

N= P. lapathifolium ssp. lapathifolium (Eur.)

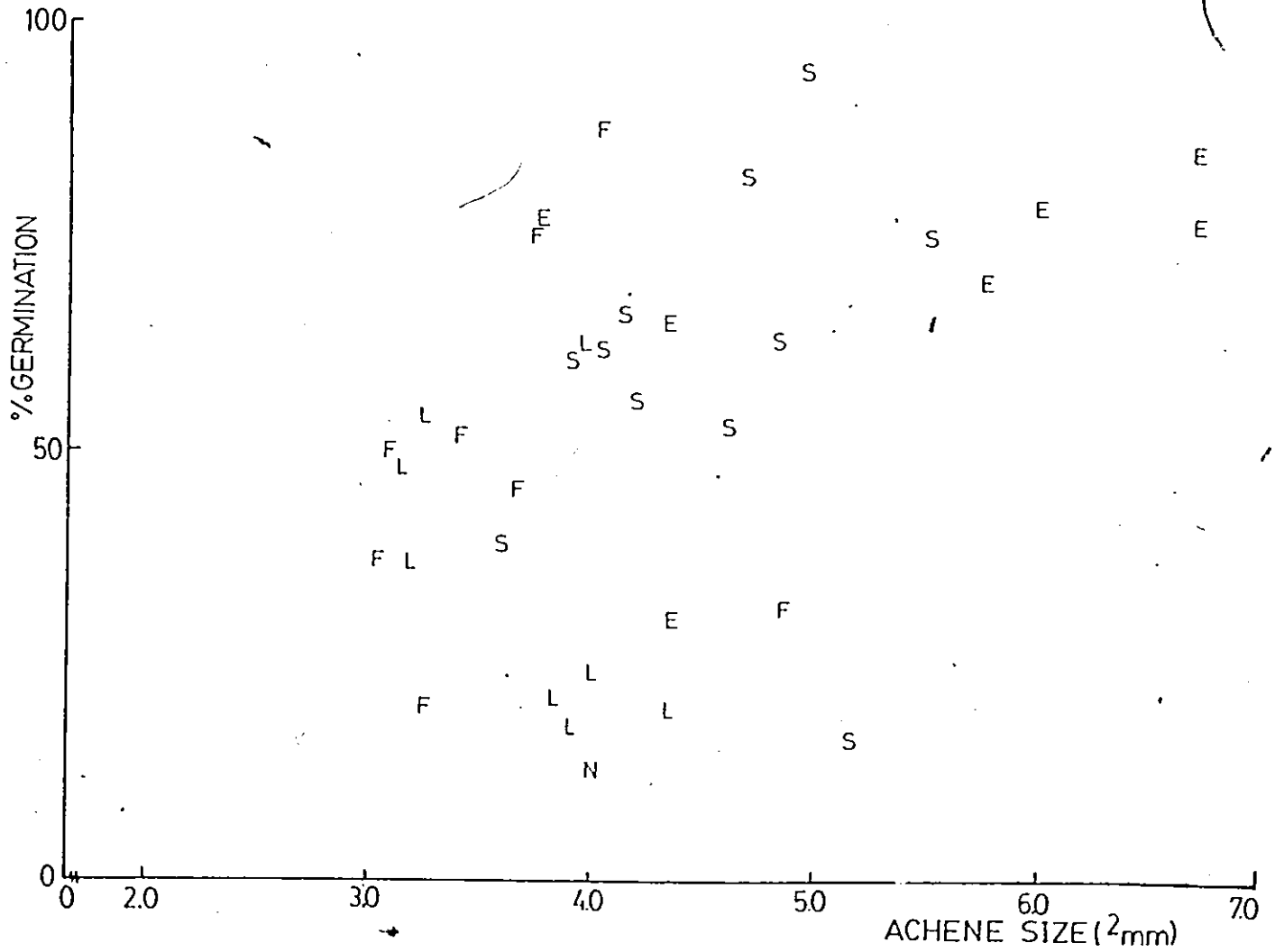

100
50
0
%GERMINATION
0
2.0
3.0
4.0
5.0
6.0
7.0
ACHENE SIZE (^{2}mm)

Figure 8: Number of weeks to flowering for North American and European populations of the P. lapathifolium complex:

Horizontal line = range;
horizontal box = standard deviation;
vertical line = mean.

Solid line= grown in greenhouses at Department of Agriculture, Ottawa.
Broken line= grown in greenhouses at University of Ottawa.

For population codes refer to Appendix A. (S= P. scabrum,
L= P. lapathifolium var. lapathifolium,
F= P. lapathifolium var. salicifolium,
E = P. lapathifolium ssp. tomentosum,
N = P. lapathifolium ssp. lapathifolium.)

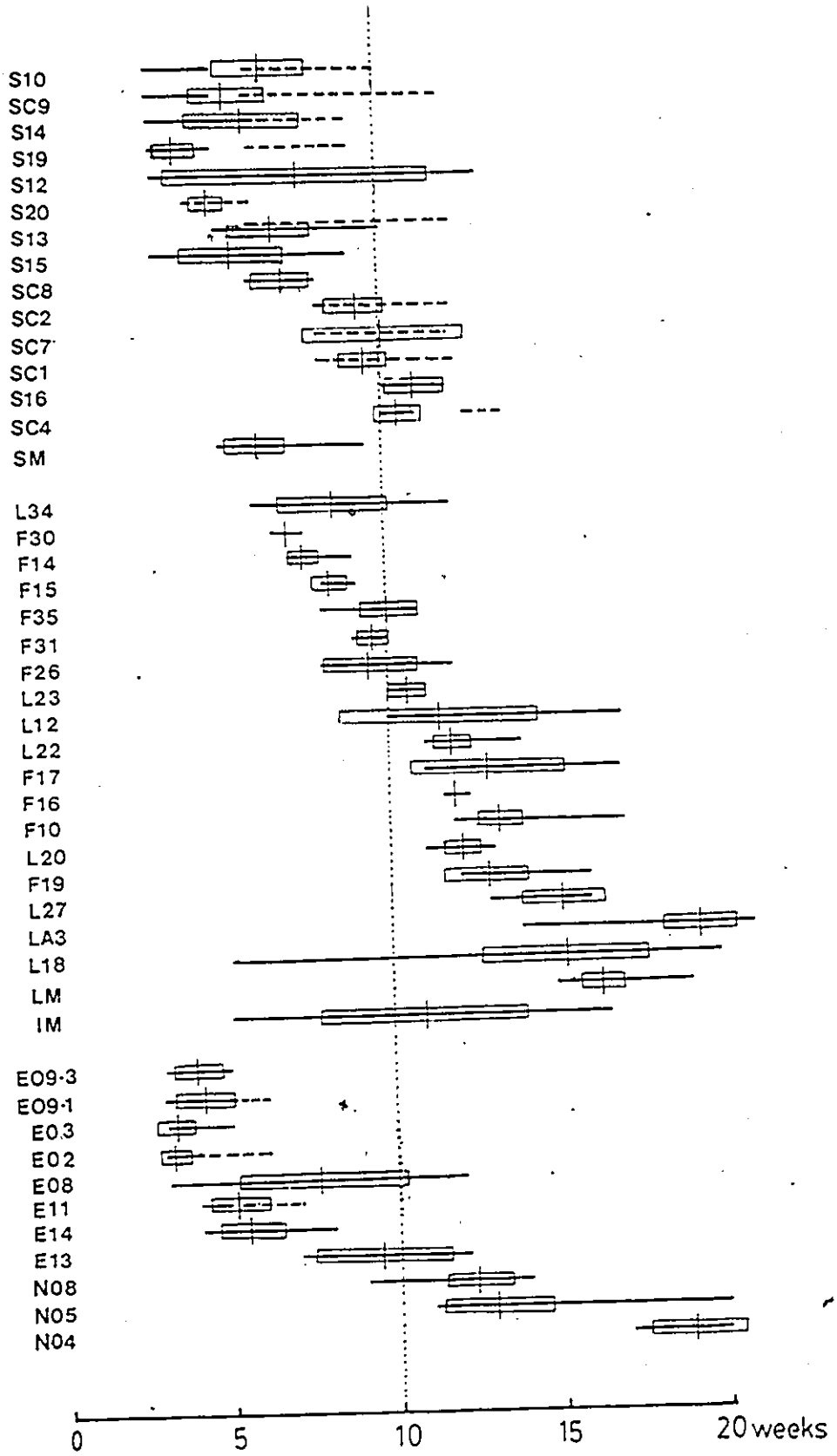
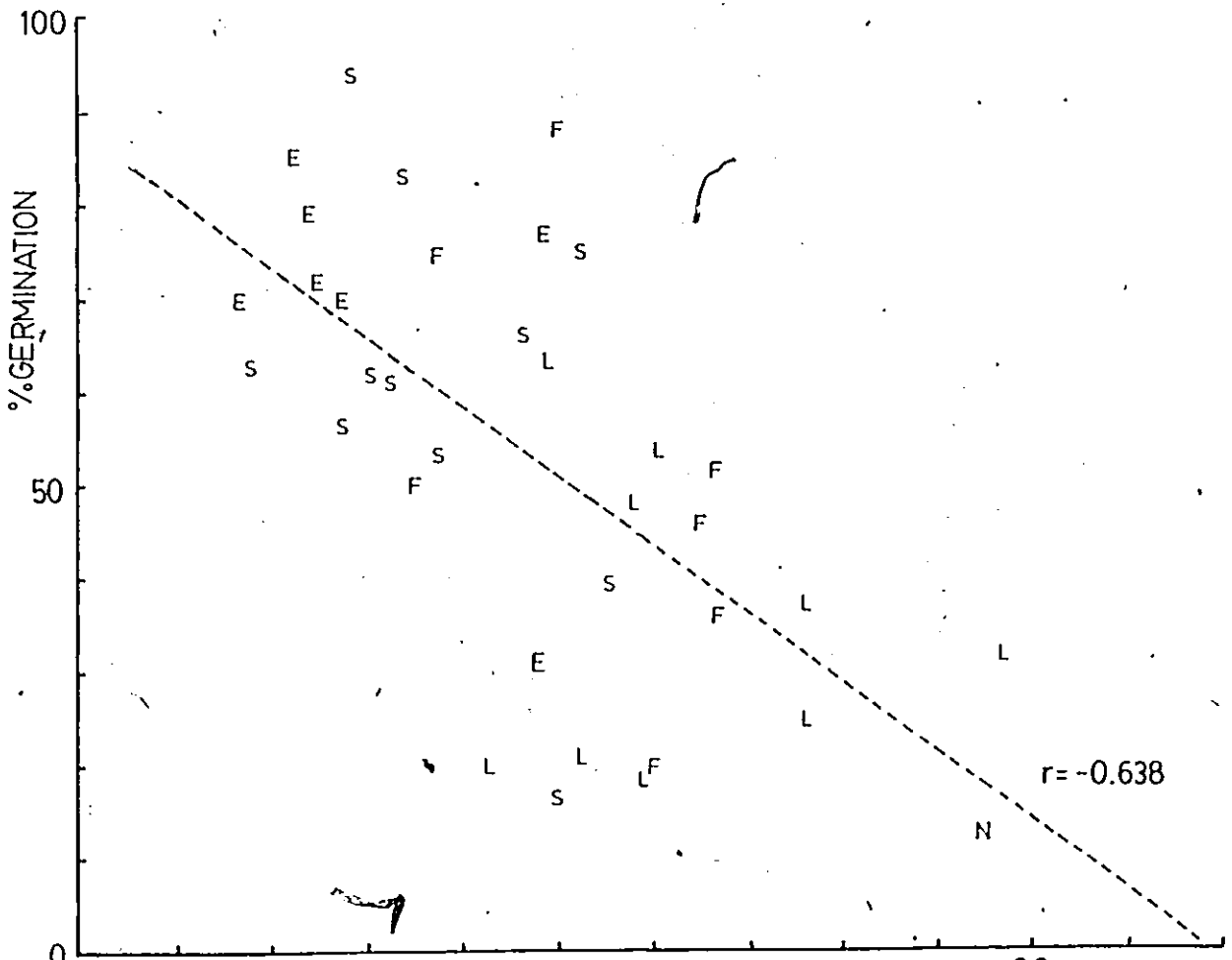

S10
SC9
S14
S19
S12
S20
S13
S15
SC8
SC2
SC7
SC1
S16
SC4
SM
L34
F30
F14
F15
F35
F31
F26
L23
L12
L22
F17
F16
F10
L20
F19
L27
LA3
L18
LM
IM
EO9·3
EO9·1
E0.3
E02
E08
E11
E14
E13
N08
N05
N04
0
5
10
15
20 weeks

Figure 9: Correlation of number of weeks to flowering (W(weeks)) with percentage of germination after stratification.
L =P. lapathifolium var. lapathifolium
F =P. lapathifolium var. salaicifolium
S =P. scabrum
E =European P. lapathifolium ssp.tomentosum
N = European P. lapathifolium ssp. lapathifolium

Line represents correlation r=0.638


100
50
0
%GERMINATION
0
10
20
W(weeks)
r= -0.638

dev.); the mean of those identified as P. lapathifolium (LM in Fig. 8) was 16.4 weeks (0.96 st. dev.) and the intermediates (IM in Fig. 8) had a large range and a mean of 13.2 weeks (3.6 st. dev.).

3.4. Cytology

3.4.1. Evaluation of Literature

Only one voucher specimen from Utrecht (Gadella & Kliphuis 1450) could be obtained for confirmation of identification. The specimen with a reported count of 2n=22 was found to be P. lapathifolium instead of P. persicaria as documented. Vouchers for the chromosome counts reported by Löve and Löve (1942a, 1982) don't appear to exist, not being in loan material examined from the University of Manitoba.

3.4.2. Experimental results

A summary of the chromosome counts obtained here is given in Table 7. Drawings of the most countable cells of P. pensylvanicum are given in Figure 10 a-c, with photographs corresponding to Figures 10 a-c in Figures 10 b-d. Counts in these cells ranged from between 2n>=37 and 2n>=42, with the majority being for 2n>=40. This is evidence for a tetraploid chromosome level, in contrast to the reports by Löve & Löve (1982).

Figure 11 represents pollen mother cells that gave counts from between 8 and 11 in dividing cells of P. scabrum and P. lapathifolium.

Success was not attained with flowers of P. persicaria.

Table 7: Polygonum lapathifolium, P. scabrum and P. pensylvanicum pollen mother cells: chromosome numbers counted in aceto-orcein squash preparations.

POPULATION CODE (see Appendix A), PLUS INDIVIDUAL PLANT NUMBER.	Count
LA3 383	11
LA5 262	11
LA5 262	8
L20 600	10
L20 602	8
SC7 452	22/2*
S10 X023	10
S13 553	11
S13 553	8
S13 553	9
S13 553	11
NP1 172	38/2
NP1 172	40/2
NP1 172	37/2
NP1 173	42/2
NP1 173	40/2

*the cells with counts "/2" indicates that the count was on an intact mother cell before meiosis, therefore, the count is for 2n chromosomes. All other counts are for cells in which division has already taken place, therefore, the count is for n chromosomes.

Figure 10: Cells of P. pensylvanicum.

a,b: Drawing and photograph of pollen mother cell of NP1 173.

c,d: Drawing and photograph (arrow) of a pollen mother cell of NP1 172.

Bar= 3um

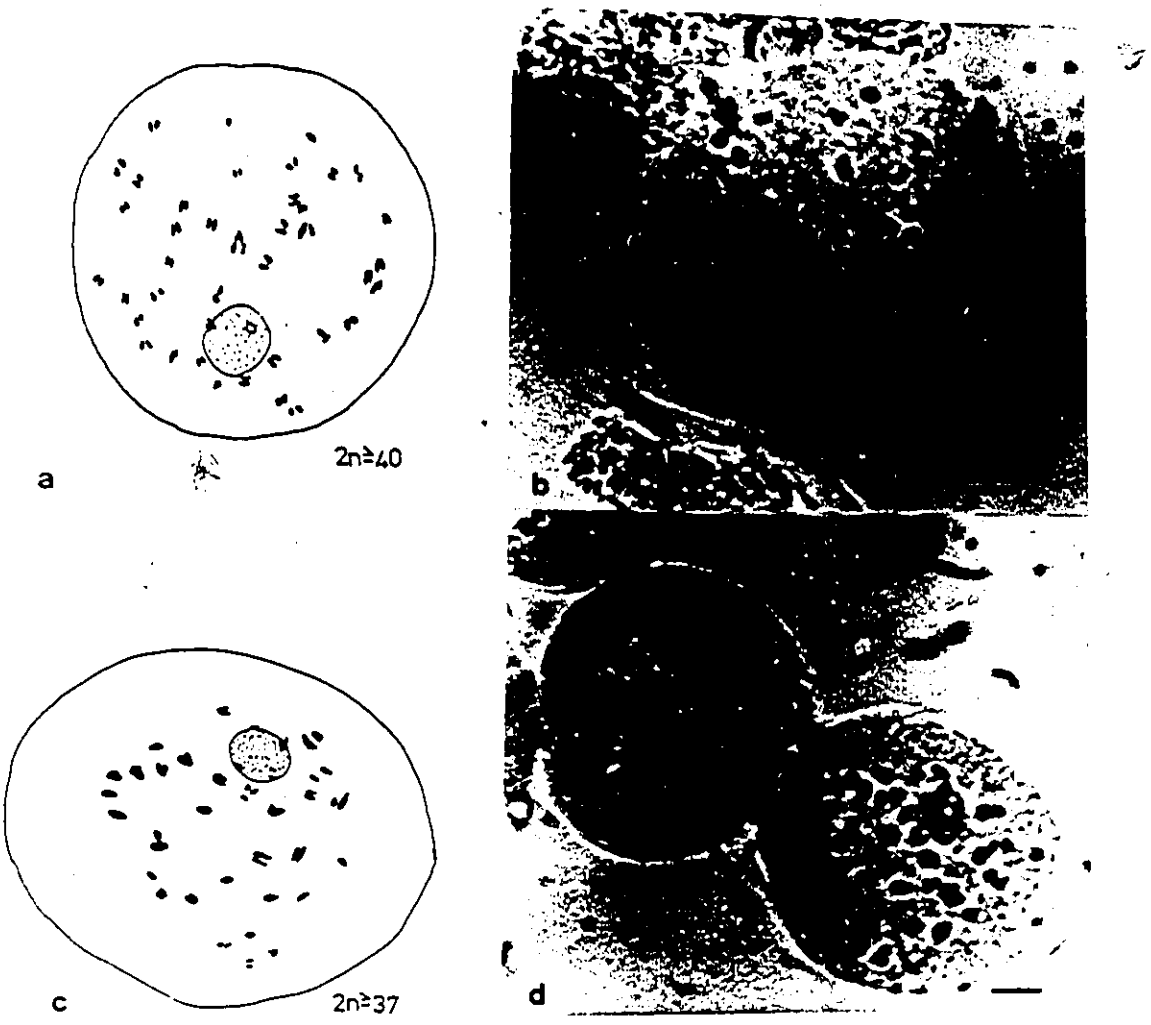
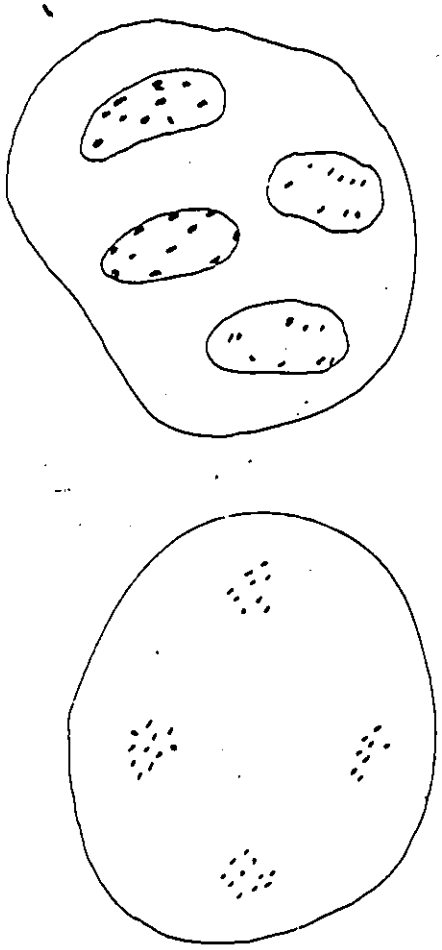

a
2n≥40
b
c
2n≥37
d

Figure 11: Cells of P. scabrum; specimen is S13 553. Drawings of observed chromosomes in dividing pollen mother cells.



3.5. Growth habit

When the progeny of the wild population plants were grown to maturity under Department of Agriculture greenhouse conditions, growth habits were different than in the wild; often strikingly so. One example of the convergence in growth habit occurred between two very different parent plants: population L18 from Rougemont, and population S13 from Black Lake, Que. (Figure 12). Population L18 parents were very tall, with inflorescences of very branched panicles, long, drooping, very pink inflorescences and strong anthocyanin in perianth and on the stem. On the other hand, S13 parents were short and very branched with many short stumpy, green axilliary spikes. Under uniform conditions, they are both very simple, unbranched plants, with similar leaf shapes and inflorescence structures.

3.6. Fruit and Leaf Morphology

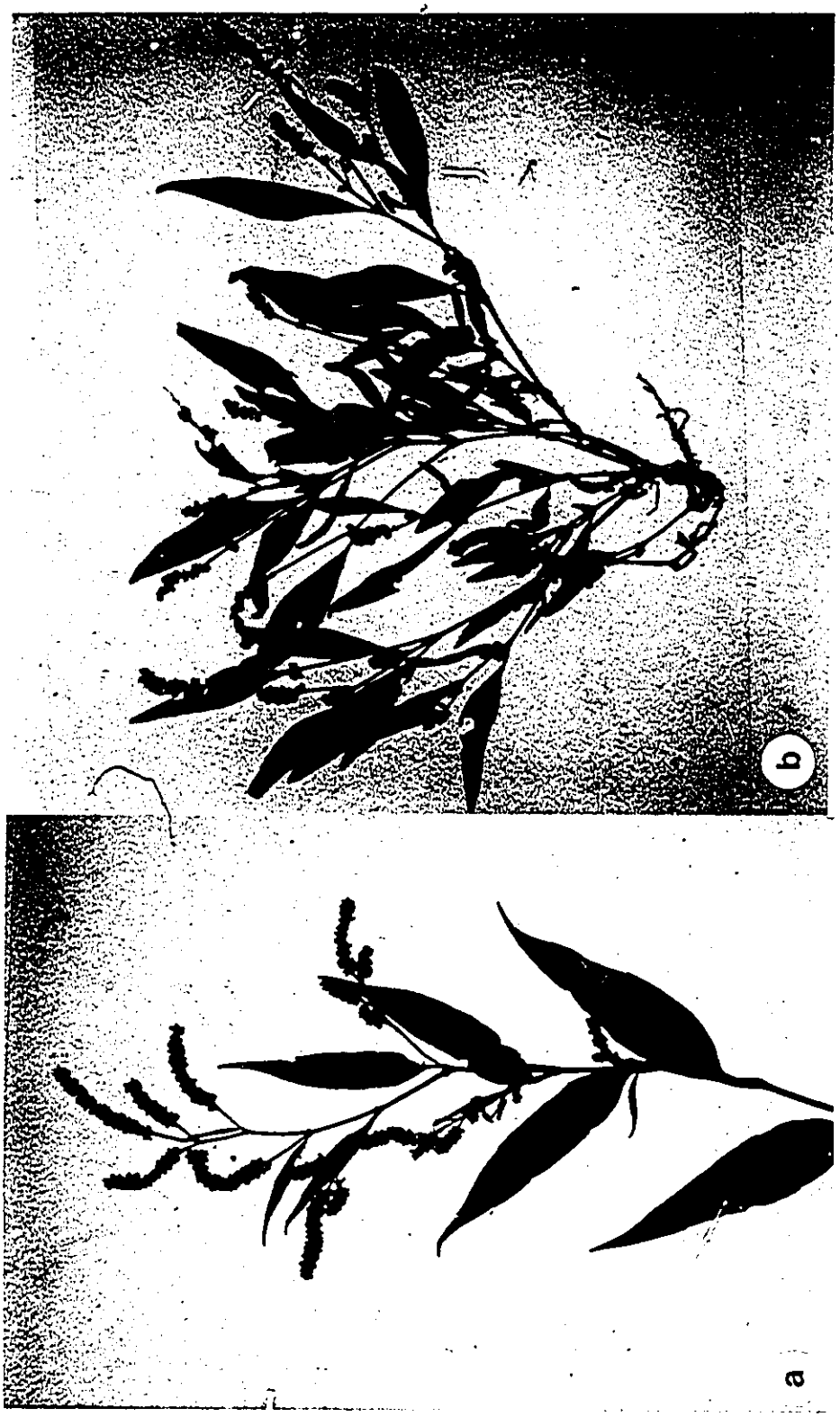
3.6.1. Univariate and Multivariate analysis

3.6.1.1. Data Set #1: P. persicaria, P. pensylvanicum and the P. lapathifolium complex (see M & M Section 2.6.2).

When the characters of the perianth and achene are plotted in pair-wise plots, P. pensylvanicum is separated from the rest of the taxa by sheer size of the perianth and achene. Figure 13 gives as an example the plot of the length and width of the perianth. In addition, character 3, presence/absence of recurved veins on the perianth, clearly separates the data set into two groups, since P.

Figure 12: Comparison of growth habit between P. lapathifolium var. lapathifolium (plant of population L18) and P. scabrum (plant of population S13). For population codes refer to Appendix A.

a: L18 wild parent
b: S13 wild parent
c: L18 progeny cultivated at Department of Agriculture greenhouses, Ottawa.
d: S13 progeny cultivated at Department of Agriculture greenhouses, Ottawa.


b
a

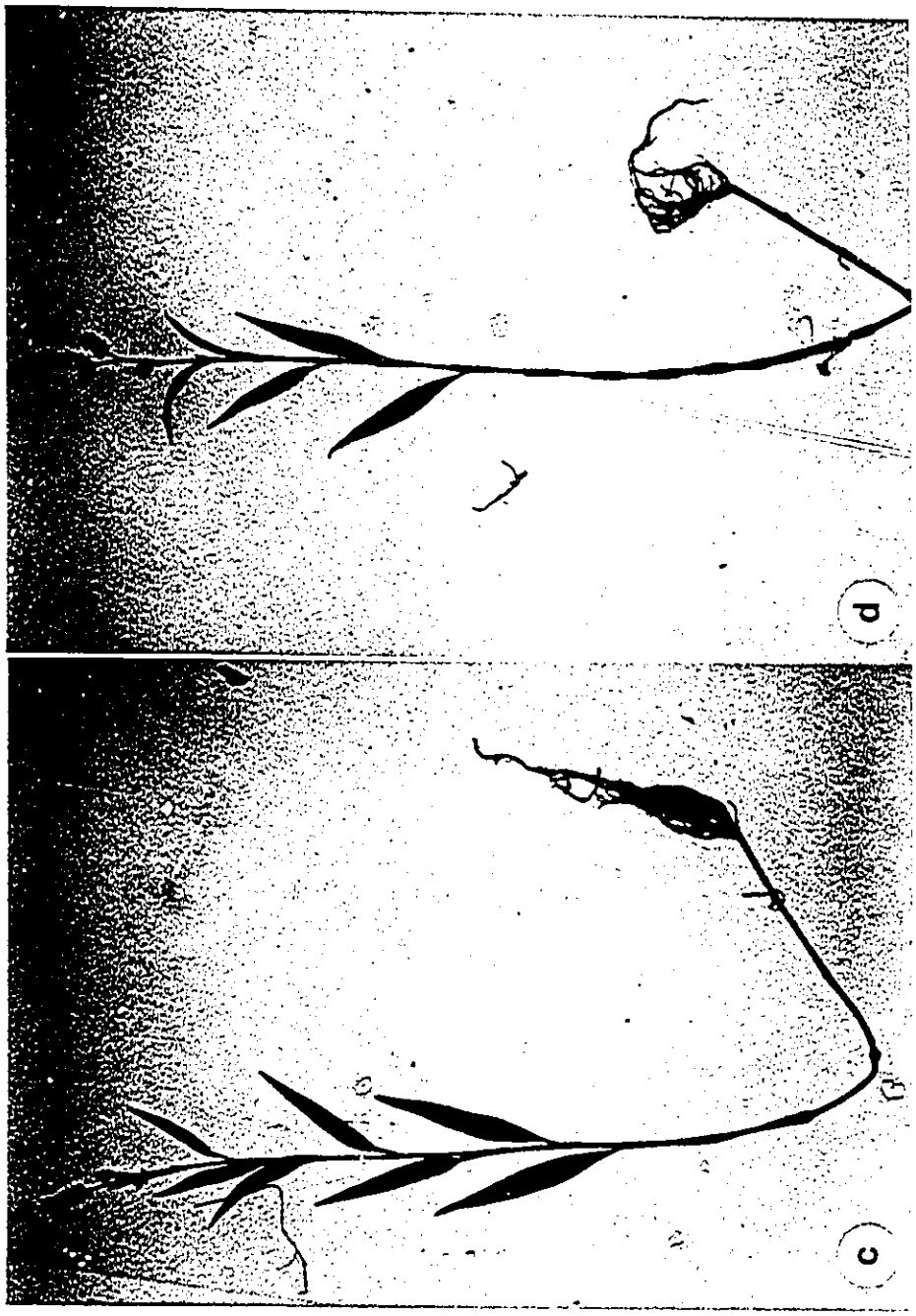

d
c

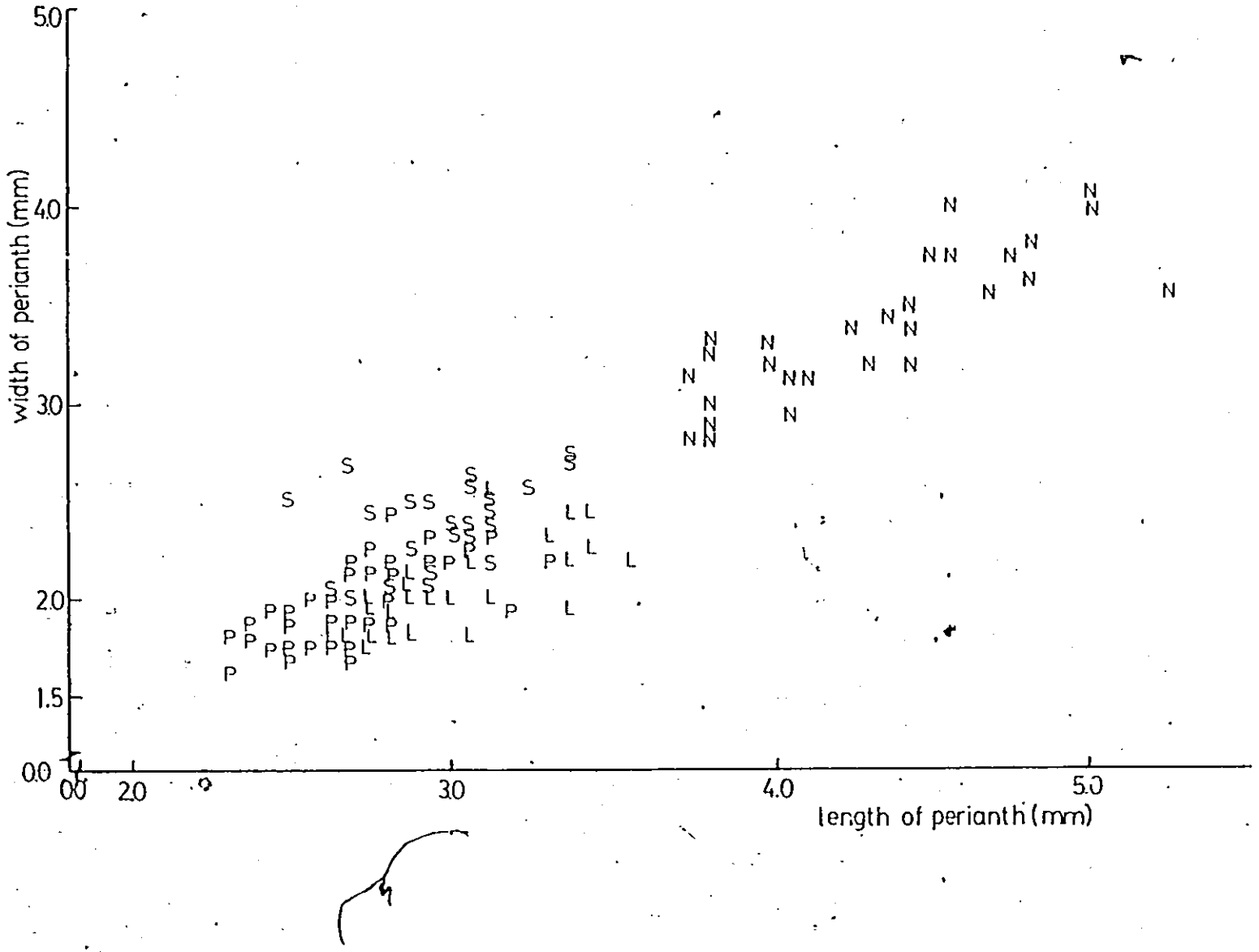
Figure 13: Plot of length vs. width of perianth for Data Set #1.

S= P. scabrum

L= P. lapathifolium

P= P. persicaria

N= P. pensylvanicum


5.0
4.0
3.0
2.0
1.5
0.0
width of perianth (mm)
0.0
2.0
3.0
4.0
5.0
length of perianth (mm)

lapathifolium and P. scabrum possess recurved veins on the perianth while P. persicaria and P. pensylvanicum do not. Multiple t-test results (see Appendix E), also reveal significant differences between P. scabrum and P. persicaria for 5 characters and between P. lapathifolium and P. persicaria for one character.

The average linkage dendrogram in Figure 14 shows that populations of P. pensylvanicum and P. persicaria are distinct from those of P. lapathifolium and P. scabrum, whereas there is some overlap between the latter two taxa. The absence of recurved veins on the perianth, and the very large achenes of P. pensylvanicum, both mentioned above, separate populations of P. persicaria and P. pensylvanicum from the other populations.

3.6.1.2. Data Set #2: The P. lapathifolium complex (see M & M Section 2.6.2.).

Univariate analysis: Phenotypic plasticity

In the ANOVA of these field and cultivated specimens, all of the characters had significantly higher among than within group variation. Table 8 shows which differences are significant between wild parents and cultivated offspring, from multiple t-tests. For the fruit size characters (length and width of perianth, length and width of achene, distance from base to widest point of achene), the means of the cultivated P. lapathifolium and most of P. scabrum were not significantly different from the means of the wild populations. An exception to this was found in five populations of P. scabrum grown in the University of Ottawa greenhouse in late summer, there-

Figure 14: Average linkage dendrogram for Data Set #1 including P. persicaria, P. lapathifolium, P. pensylvanicum and P. scabrum, using Gower coefficient. Percentages of total distance given. In four cases individual plants did not group with the originally determined species: L= P. lapathifolium in P. scabrum group & S= P. scabrum in P. lapathifolium group.

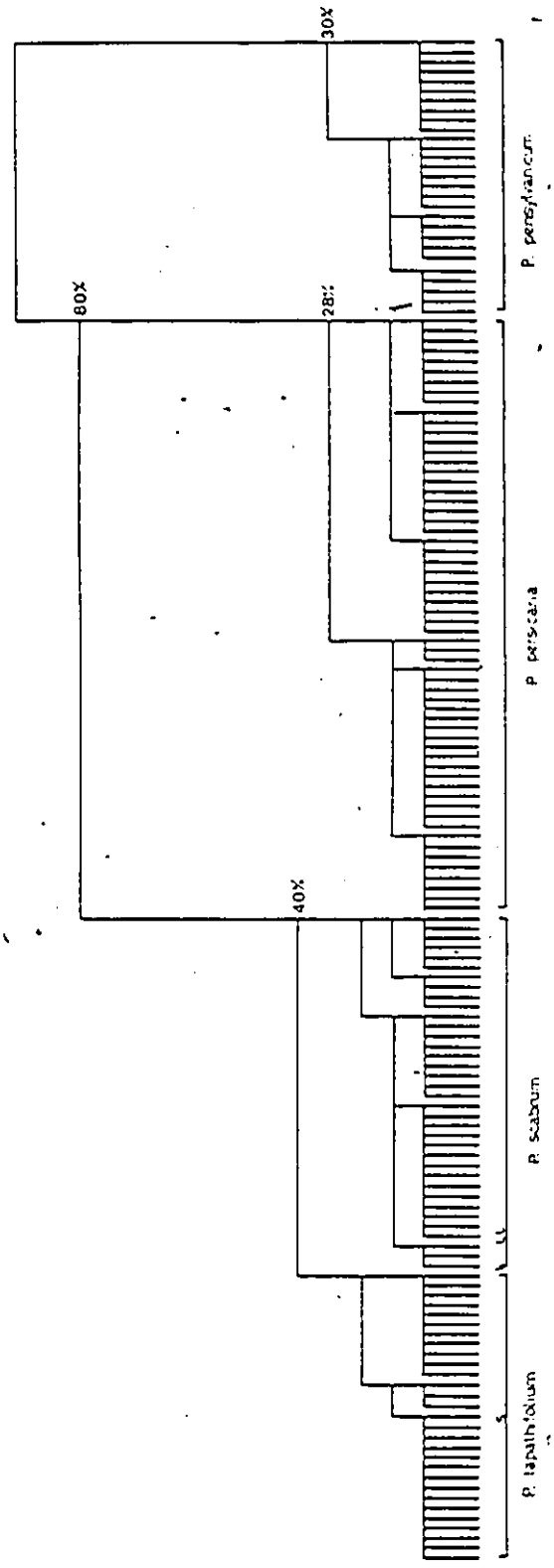

30X
80X
28X
40X
P. persicaria
P. scabrum
P. lapathifolium

Table 8: Dataset #2: Multiple comparison t-test results: wild vs. cultivated plants for P. scabrum, P. lapathifolium var. lapathifolium and P. lapathifolium var. salicifolium **= significant at p=0.01, n.s.= not significant.

Character	P. lapathifolium var. lapathifolium	P. lapathifolium var. salicifolium	P. scabrum
1	n.s.	n.s.	n.s.
2	n.s.	n.s.	n.s.
4	**	**	n.s.
5	n.s.	n.s.	n.s.
6	n.s.	n.s.	n.s.
7	n.s.	n.s.	n.s.
8	n.s.	**	n.s.
9	**	**	n.s.
10	**	**	n.s.
11	n.s.	n.s.	**
12	n.s.	n.s.	n.s.
13	n.s.	n.s.	n.s.
14	n.s.	n.s.	**
15	n.s.	n.s.	**
16	**	**	n.s.

fore under different greenhouse conditions. These plants exhibited extreme phenotypic plasticity, being short and spindly with significantly larger achenes than the other cultivated P. scabrum plants. These were not used in the subsequent taxon comparisons. Colour characters (7-9) were not stable in cultivation. The size of the fourth leaf is also significantly different in P. scabrum cultivated specimens vs. that in wild plants. The height is significantly different between wild and cultivated specimens of both varieties of P. lapathifolium, although not so for P. scabrum. There was a significantly greater number of glands in cultivated than in wild plants of both varieties of P. lapathifolium.

Inter-taxon variation

Table 9 compares the means of the taxa that did not have significantly different means for wild and cultivated plants. Polygonum lapathifolium var. lapathifolium and var. salicifolium differed significantly in length of achene (character #5) for both wild and cultivated plants, and, moreover, these two were significantly different from P. scabrum for width of perianth (#2), length (#5) and width (#6) of achene and distance from base of the achene to widest point (#7).

The colour characters and vegetative characters were less diagnostic. Variety salicifolium showed significant differences between wild and cultivated for all colour characteristics, while var. lapathifolium had significant differences in the intensity and extent of pink but not the intensity of green in the inflorescence.

Table 9: Inter-taxon comparisons from multiple t-test (see Appendix E). ** = significant at p=0.01.
L = P. lapathifolium var. lapathifolium - wild
Y = P. lapathifolium var. lapathifolium - cultivated
F = P. lapathifolium var. salicifolium - wild
Z = P. lapathifolium var. salicifolium - cultivated
S = P. scabrum - wild
X = P. scabrum - cultivated

Char.	group	L	Y	F	Z	S	X
1	L	-	n.s.	n.s.	n.s.	n.s.	n.s.
	Y		-	n.s.	n.s.	n.s.	n.s.
	F			-	n.s.	n.s.	n.s.
	Z				-	n.s.	n.s.
	S					-	n.s.
2	L	-	n.s.	n.s.	n.s.	**	n.s.
	Y		-	n.s.	n.s.	**	**
	F			-	n.s.	**	**
	Z				-	**	**
	S					-	n.s.
5	L	-	n.s.	**	**	**	**
	Y		-	**	**	**	**
	F			-	n.s.	**	**
	Z				-	**	**
	S					-	n.s.
6	L	-	n.s.	n.s.	n.s.	**	**
	Y		-	n.s.	n.s.	**	**
	F			-	n.s.	**	**
	Z				-	**	**
	S					-	n.s.
7	L	-	n.s.	n.s.	n.s.	**	**
	Y		-	n.s.	n.s.	**	**
	F			-	n.s.	**	**
	Z				-	**	**
	S					-	n.s.
12	L	-	n.s.	n.s.	n.s.	n.s.	n.s.
	Y		-	n.s.	n.s.	n.s.	n.s.
	F			-	n.s.	n.s.	n.s.
	Z				-	n.s.	n.s.
	S					-	n.s.
13	L	-	n.s.	n.s.	n.s.	n.s.	n.s.
	Y		-	n.s.	n.s.	n.s.	n.s.
	F			-	n.s.	n.s.	n.s.
	Z				-	n.s.	n.s.
	S					-	n.s.

Cultivated Polygonum scabrum specimens were not significantly different than the wild for any of these characters; none of the leaf characters were taxonomically helpful. As mentioned, the length and width of the fourth leaf showed a significant change in size in the cultivated P. scabrum leaves, and for the first leaf there was no significant difference between any of the North American groups.

Multivariate analysis

The first two components, representing a total of 56% of the variation, from a principal components analysis on the set of populations in Data Set #2 is illustrated in Figure 15. It shows that the populations of this complex separate into 2 groups. One is composed of P. scabrum (North America) and P. lapathifolium ssp. tomentosum (Europe); the other of P. lapathifolium var. lapathifolium (North America) and P. lapathifolium ssp. lapathifolium (Europe). There is one population of var. salicifolium, however, that clusters with the first group. In addition, the pattern is contiguous, not separating into two distinct groups. The characters contributing the greatest percentage of variance to the first component axis are all fruit size characters: 2, 5, 6 and 7.

3.6.1.3. Data Set #3: Mixed population of the P. lapathifolium complex.

The principal components analysis results on this data set (Figure 16) shows that the plants originally identified as P. lapathifolium var. lapathifolium and P. scabrum did sort into

Figure 15: Principal components analysis on OTUs of Data Set #2: Plot of first two component axes. Symbols represent population means.
L,l= P. lapathifolium var. lapathifolium
F,f= P. lapathifolium var. salicifolium
S,s= P. scabrum
c= European P. lapathifolium ssp. tomentosum
n= European P. lapathifolium ssp. nodosum

Upper case letters refer to wild populations, lower case letters refer to cultivated populations.

Two populations of P. lapathifolium var. salicifolium grouped with P. scabrum populations; these symbols circled: see text.

Percentage of variation accounted for by each axis: first principal component: 41%
second principal component: 15%

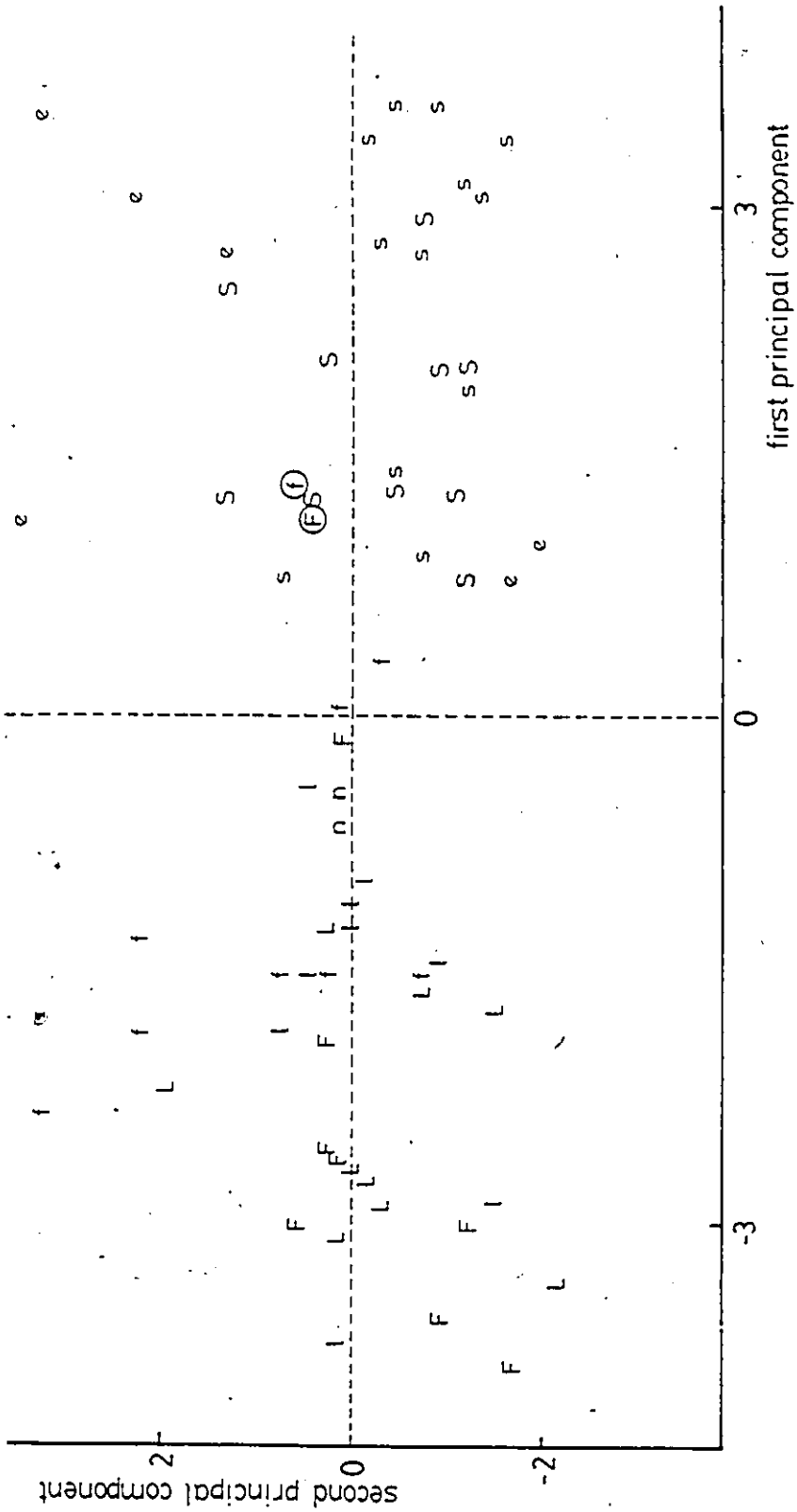
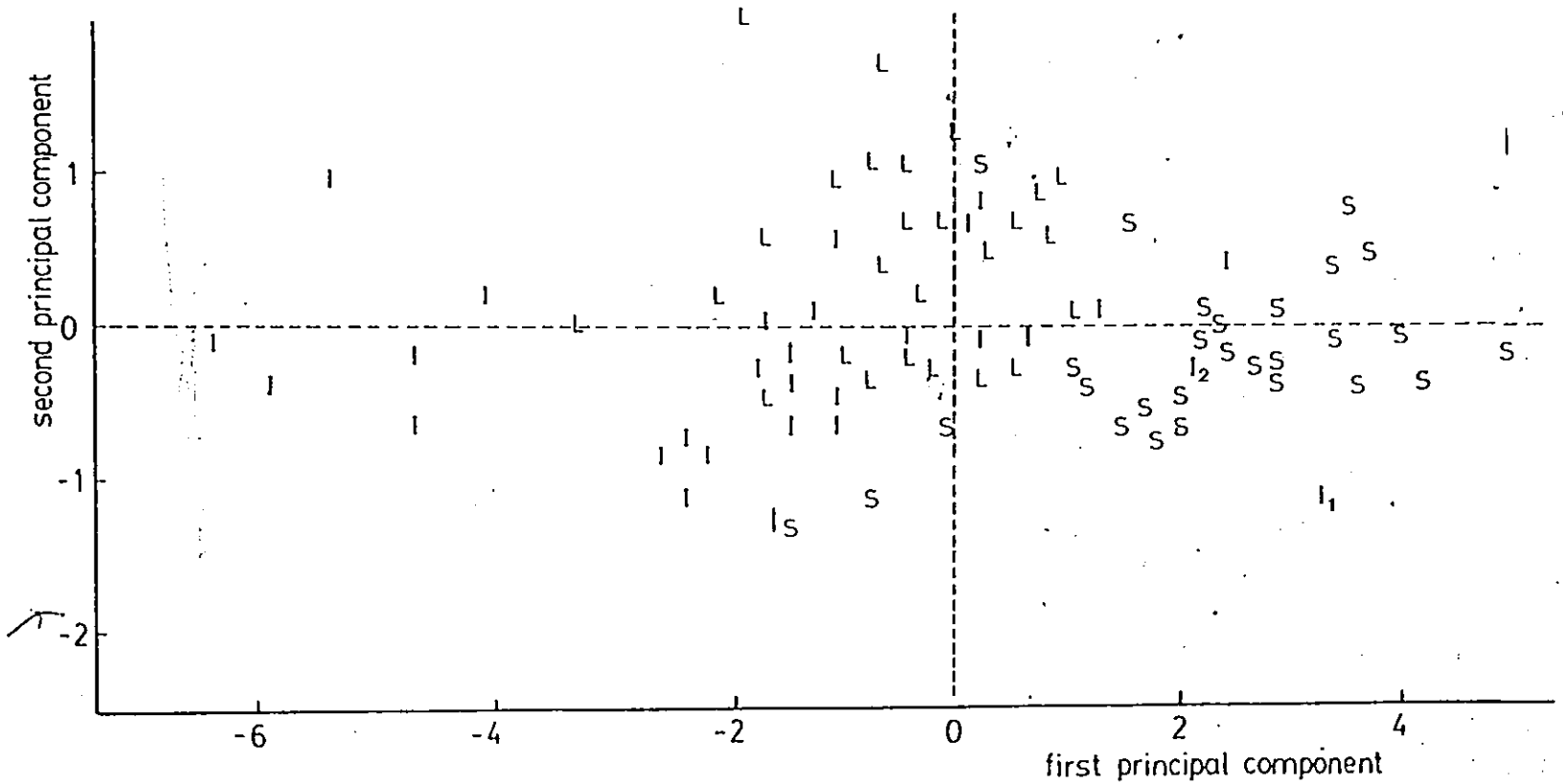

first principal component
second principal component
3
0
-3
2
0
-2

Figure 16: Principal components analysis on OTUs of Data Set #3. Plot of first two component axes. Symbols represent individual plants.

S = P. scabrum.
L = P. lapathifolium var. lapathifolium
I = Intermediates

#1 and #2: intermediate plants possessing different electrophoretic genotype; see Section 3.8.3.

Percentage of variation represented on each axis: first principal component: 41%
second principal component: 16%


second principal component
1
0
-1
-2
-6
-4
-2
0
2
4
first principal component

separate groups. The plants identified a priori as 'intermediates' did not fall in the middle of these two groups, but scattered over the entire plot, showing that these are not simply hybrids of two parent taxa. This particular group of plants were identified as intermediate because they were not strikingly glandular; had neither long lax inflorescences nor short stumpy ones; and had neither extremely pink nor particularly green perianths (in growth habit the plants were all very similar). Since the first component axis (accounting for 41% of the total variation) reflects the size of the perianth and achenes, it appears that this size varies quite widely among individuals that I identified as intermediate.

3.7. Flavonoid Analysis

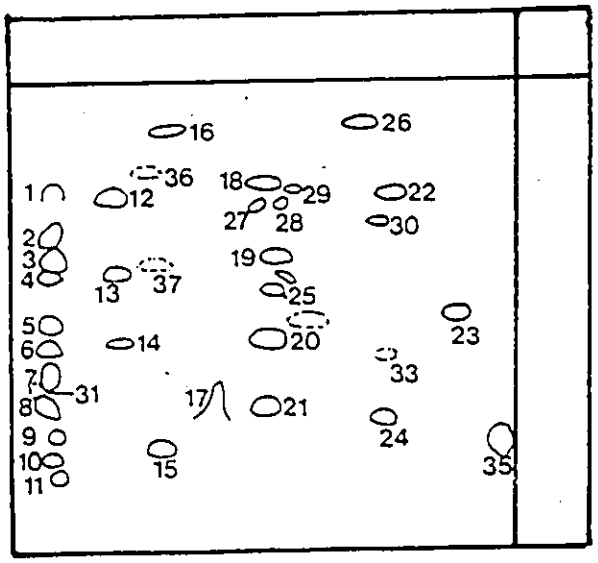
The general pattern observed on the thin layer chromatography plates is shown in Figure 17. Thirty-six flavonoids were recognized in specimens of the P. lapathifolium complex, and numbered as in Figure 17a. When P. persicaria and P. pensylvanicum were also considered, a total of 44 compounds were recognized (additional numbered compounds in Figure 17b). Table 10 lists the characteristics and Rf values of each of the compounds.

Of the 44 compounds in total, five (14%) were found in all 26 extracts encompassing all species. Twelve (33%) were found in all specimens of the P. lapathifolium complex. In many cases, overlapping of the compounds occurred, particularly compounds numbered 9-11. The chromatographic patterns of North American P. lapathifolium and P. scabrum were virtually identical (Figure 18).

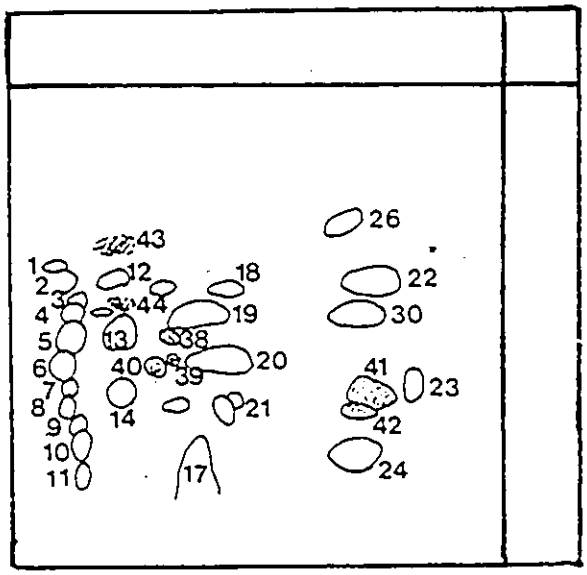
Figure 17: Principal compounds numbered on two-dimensional TLC plates in

a: P. lapathifolium and P. scabrum, and

b: P. persicaria and P. pensylvanicum.


16
26
36
18
29
22
1
12
27
28
30
2
3
4
19
13
37
25
5
6
14
20
23
33
7
31
17
21
8
24
9
10
15
35
11

a


43
26
1
12
18
22
2
3
44
4
19
30
5
13
38
6
40
39
20
41
23
7
8
14
21
42
9
10
17
24
11

b

Table 10: Characteristics of unhydrolyzed flavonoid compounds in two-dimensional thin-layer chromatography of Polygonum species. K=kaempferol, Q=quercetin, PA=phenolic acid, NA=naringenin glycoside I=isorhamnetin and ?=unknown.

Compound number	Colour	rf organic	rf aqueous	Aglycone id
1	green	.63	.03	K
2	yellow/green?	.55	.03	I?
3	yellow/orange	.50	.03	Q
4	green	.43	.03	K
5	yellow/green	.38	.03	I
6	green	.37	.03	K
7	yellow/green	.33	.03	I
8	orange	.30	.03	Q
9	orange	.20	.03	?
10	dull y/gr	.15	.03	?
11	dull y/gr	.08	.03	?
12	green	.60	.13	K
13	orange	.47	.13	Q
14	yellow/orange-red	.22	.13	?
15	yellow/orange	.10	.13	Q
16	blue	.77	.38	PA
17	blue	.22	.32	PA
18	green	.58	.50	K
19	yellow	.47	.50	Q
20	yellow	.30	.45	Q
21	orange	.18	.43	Q
22	blue	.62	.75	PA
23	blue	.37	.76	PA
24	white	.17	.70	?
25	green	.43	.50	K
26	blue	.83	.78	PA
27	blue	.53	.45	PA
28	yellow	.55	.52	?
29	blue	.58	.60	PA
30	blue	.60	.78	PA
31	purple	.18	.00	?
32	yellow	.50	.75	Q
33	purple	.35	.50	?
34	white	.65	.03	?
35	white	.17	.95	?
36	g/blue	.72	.25	PA
37	orange	.28	.45	Q
38	yellow	.27	.38	Q
39	yellow	.27	.37	Q
40	yellow	.25	.35	Q
41	black	.32	.75	NA
42	green	.27	.68	K
43	green	.6?	.13	K
44	orange	.50	.13	Q

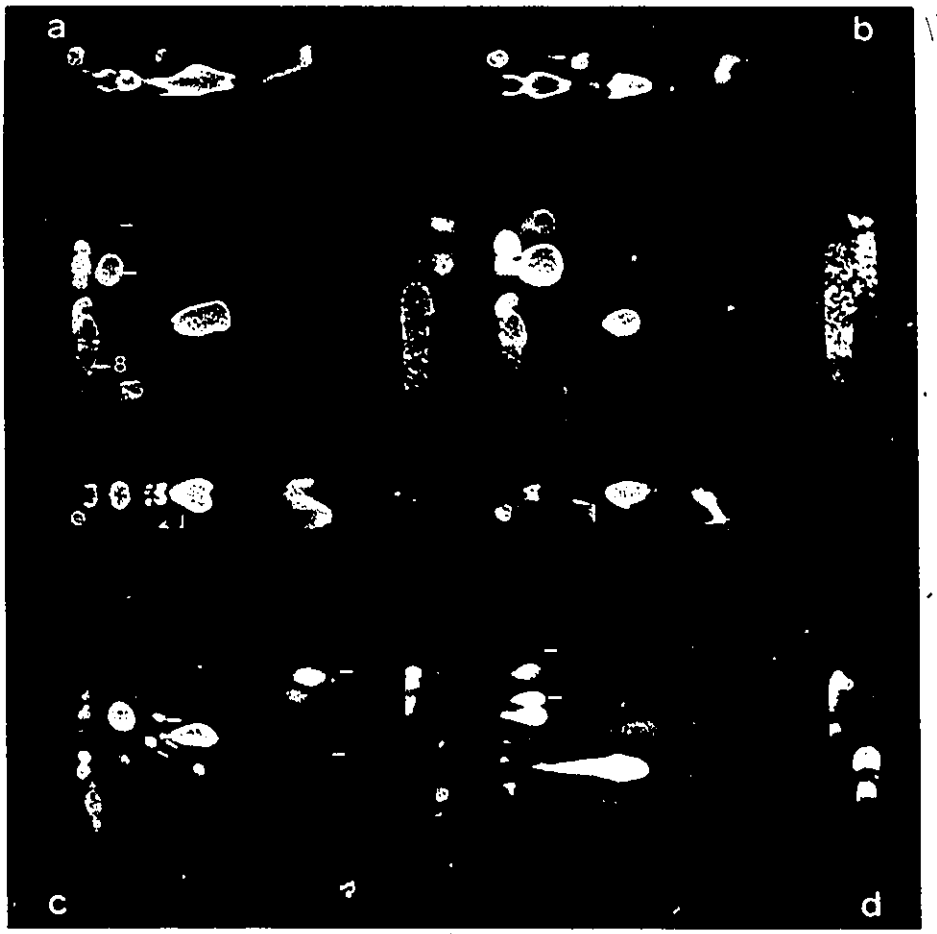
Figure 18: Two-dimensional TLC plates under long-wave uv light.
a: P. scabrum (SC4#308)
b: P. lapathifolium (LA3#378)

tentative identities of
marked spots: green=kaempferol-3-O-glucoside
yellow=quercetin-3-O-glucoside
#8=quercetin-3-O-glucoside-2''-gallate

c: P. persicaria (P10#530)
d: P. pensylvanicum (NP5#392)

marked spots= characteristic compounds for each of P. persicaria and P. pensylvanicum.

For population codes see Appendix A.


a
b
c
d

After spraying with the flavone reagent, the compounds fluoresced green (kaempferol), orange (quercetin), yellow-green (isorhamnetin); in many cases, however, isorhamnetin was difficult or impossible to distinguish because of the overlapping of quercetin and kaempferol glycosides in the extraction. Compounds 1-11 did not migrate in the organic solvent, suggesting that they are aglycones and/or monoglycosides (F.W. Collins, pers. comm.). The migration of compounds 12-15 suggests that they may be diglycosides, and the rest of the flavonoids possessed higher levels of glycosylation. Eight of the compounds fluoresced blue with the flavone reagent under uv light; these are phenolic acids, migrating substantially in both the aqueous and organic solvents (C. Nozzolillo, pers. comm.).

The patterns of P. persicaria and P. pensylvanicum are shown in Figure 17b and 18c and d, and vary slightly from those found in the P. lapathifolium complex. Five extra compounds were found in P. persicaria; one of these was black in colour in uv light with the flavone reagent, an indication that it is related to naringin. One of the two samples of P. pensylvanicum had two extra compounds, a kaempferol glycoside and a quercetin glycoside, that were not present in any other preparations.

Detailed identification of the compounds was not performed, but those that migrated with the flavonoid standards were able to be tentatively identified. Compound #22 migrated with chlorogenic acid, most reliably seen in the preparations of P. persicaria. Compound #13 co-chromatographed with quercetin-3-O-glucoside and quercetin-3-O-rhamnoside; #8 with quercetin-3-O-glucoside-2"-gallate and quer-

cetin-3-O-rhamnoside-2"-gallate; #12 with kaempferol-3-O-glucoside. Detection of kaempferol-3-O-glucoside-2"-gallate may not have been possible because the standard co-migrates with quercetin-3-O-glucoside-2"-gallate.

The cluster analysis to determine whether the presence and absence of the minor flavonols was diagnostic for taxa revealed no separation of taxa within the P. lapathifolium complex on the basis of presence or absence of flavonoids (Figure 19), but did show the complex as distinct from P. persicaria and P. pensylvanicum.

Anthocyanin is produced in young seedlings of almost all populations of the P. lapathifolium complex when grown in light (Figure 20). In P. scabrum this production usually (in all cases here) stops before maturity, but in P. lapathifolium the anthocyanin is often persistent. A group of populations of P. lapathifolium, including four North American (L18, L22, L12, and L13), one European (N05) and the Japanese (J01) populations possessed a striking anthocyanin pattern in the stem (Figure 21). Other populations simply have a ring of anthocyanin around the top of each internode (e.g. population F18). These patterns are also obvious in many herbarium specimens.

3.8. Electrophoretic results

3.8.1. Banding patterns

3.8.1.1. P. lapathifolium and P. scabrum (2n=22)

The patterns of the fifteen enzyme systems for each of the taxa are shown in Figure 22.

Figure 19: Dendrogram of presence and absence of flavonoid compounds using Jaccard coefficient.

L= P. lapathifolium var. lapathifolium

F= P. lapathifolium var. salicifolium

S= P. scabrum

Np= P. pensylvanicum

P= P. persicaria

E= European P. lapathifolium ssp. tomentosum

N= European P. lapathifolium ssp. lapathifolium

110

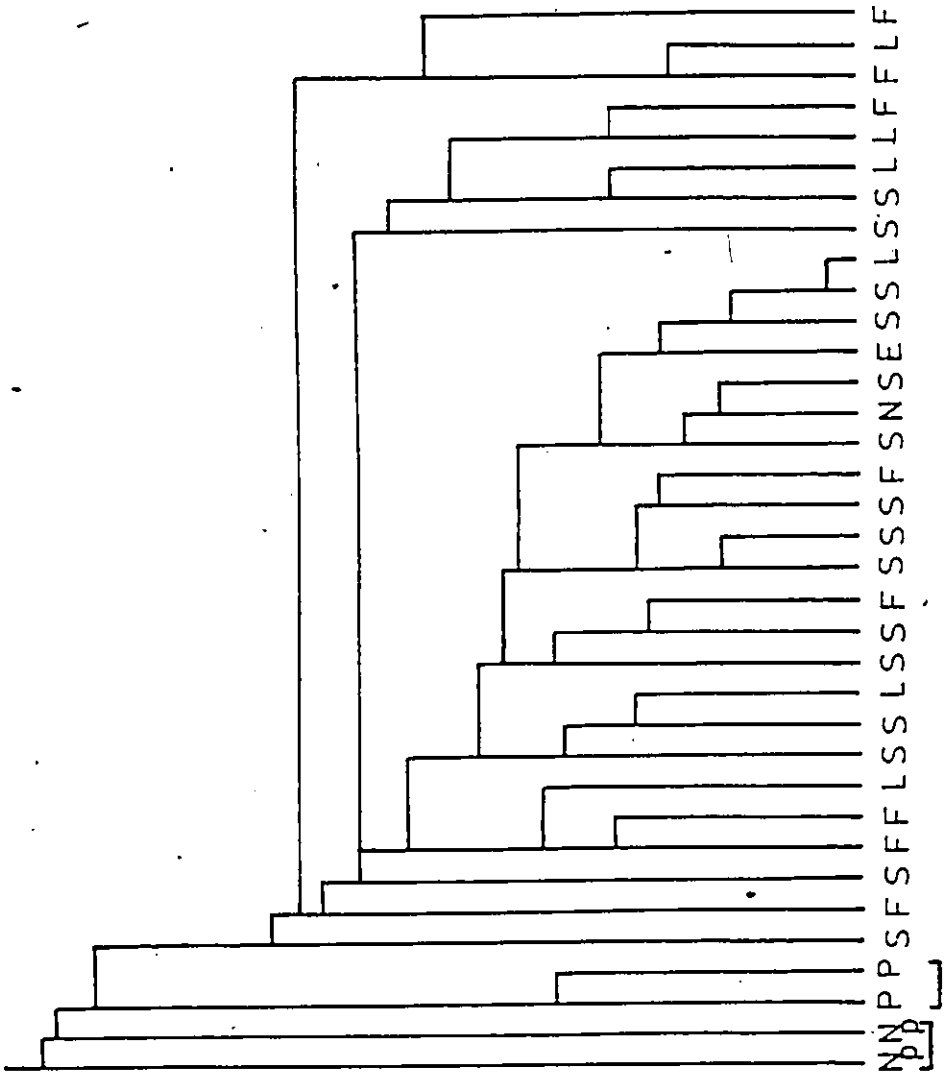


Figure 20: Seedlings at 10 days of age.

A. P. scabrum SC2#256 (25X)

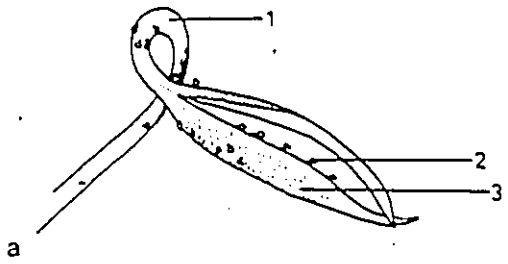
1. Very sparse glands on hypocotyl
2. Sparse, colourless gland-tipped hairs
3. Reddish tinge on otherwise dark-green cotyledons

B. P. lapathifolium LA5#262 (40X)

Almost without glands; glands very small and clear.

4. Deep red colour in cells
5. Some seedlings only red at tip of cotyledons

For population codes see Appendix A.


1
2
3
a

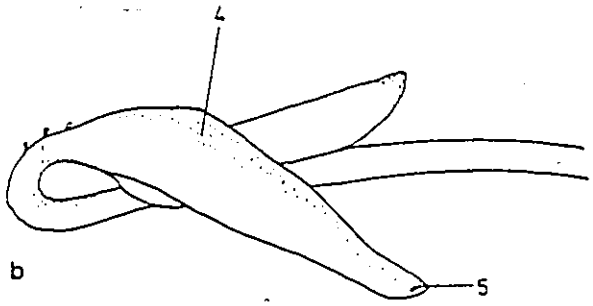
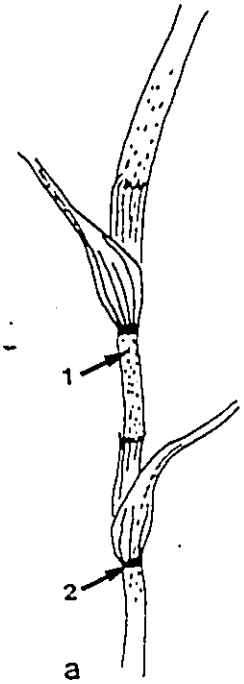

4
b
5

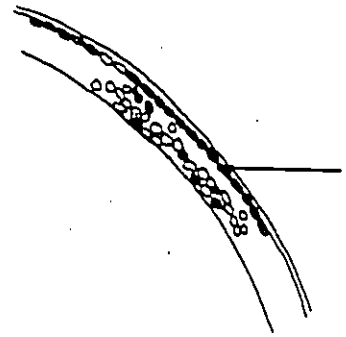
Figure 21: Anthocyanin pattern in stem of several populations of P. lapathifolium: L18, L22, E05, J01. For population codes refer to Appendix A.

a: General sketch of anthocyanin position.
Pointers to areas of red anthocyanin.
1. spots in internodes
2. ring just under node

b: Cross-section of stem at an internodal point through a red spot.
pointer: dark cells pigmented (red)

c: Cross-section of stem at internodal point through a red spot where stem pink-coloured.
Pointers:
1. some red cells in epidermal layer
2. rest of epidermal cells (2 layers) green
3. 2 or 3 very photosynthetic layers
4. 2 layers of thick-walled cells
5. photosynthetic layer with bundles
6. hexagonal pigmented layer


1
2
a


b

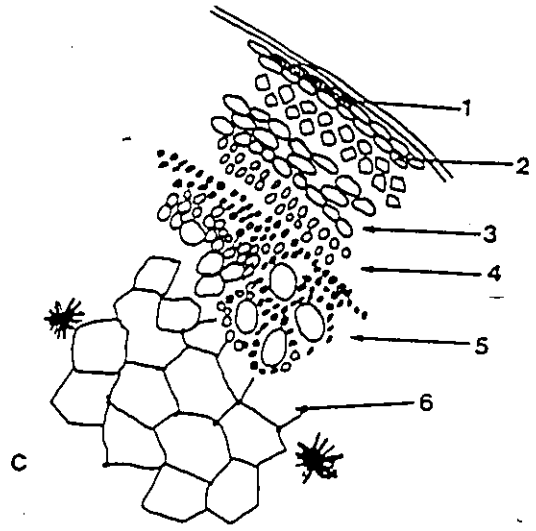
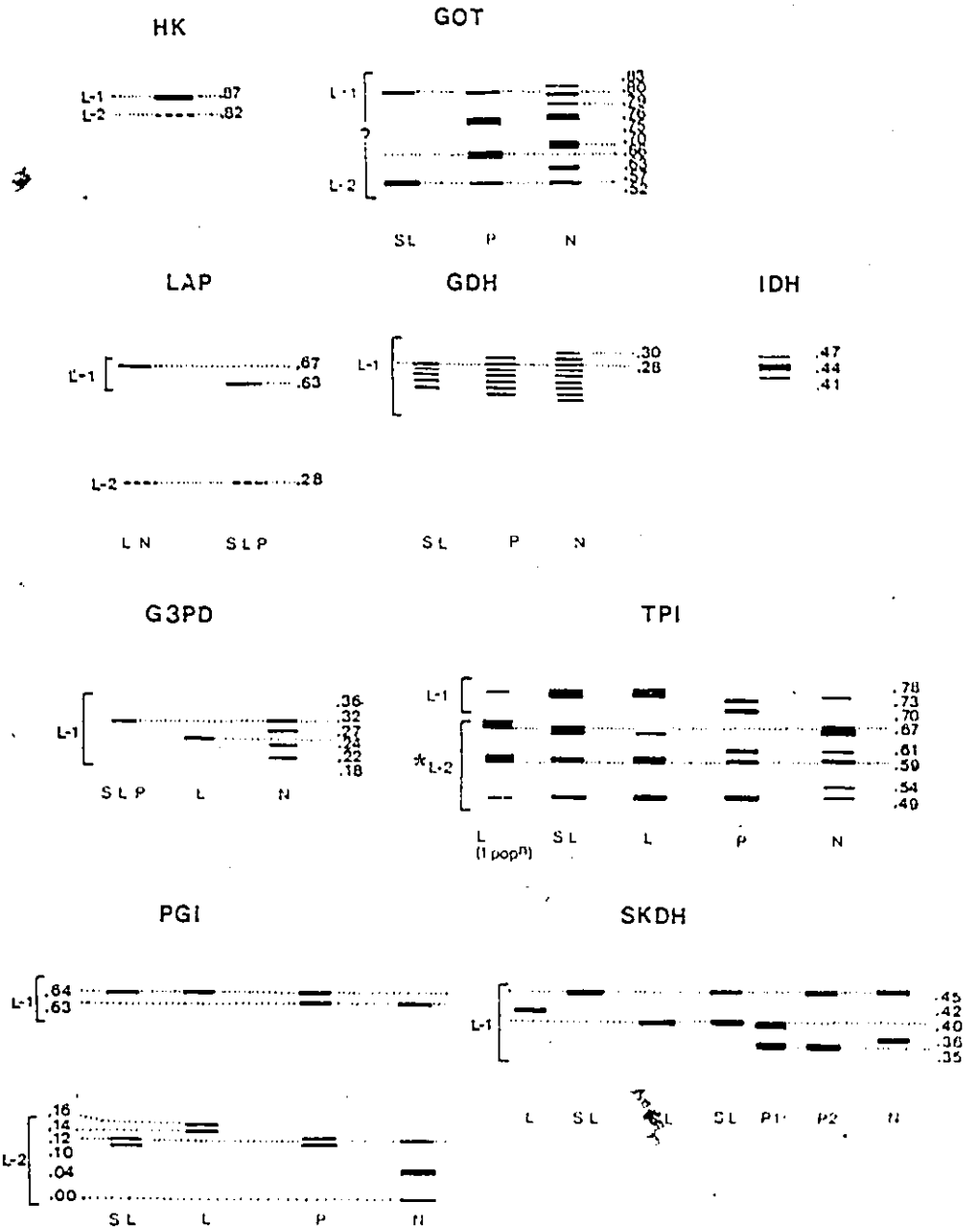
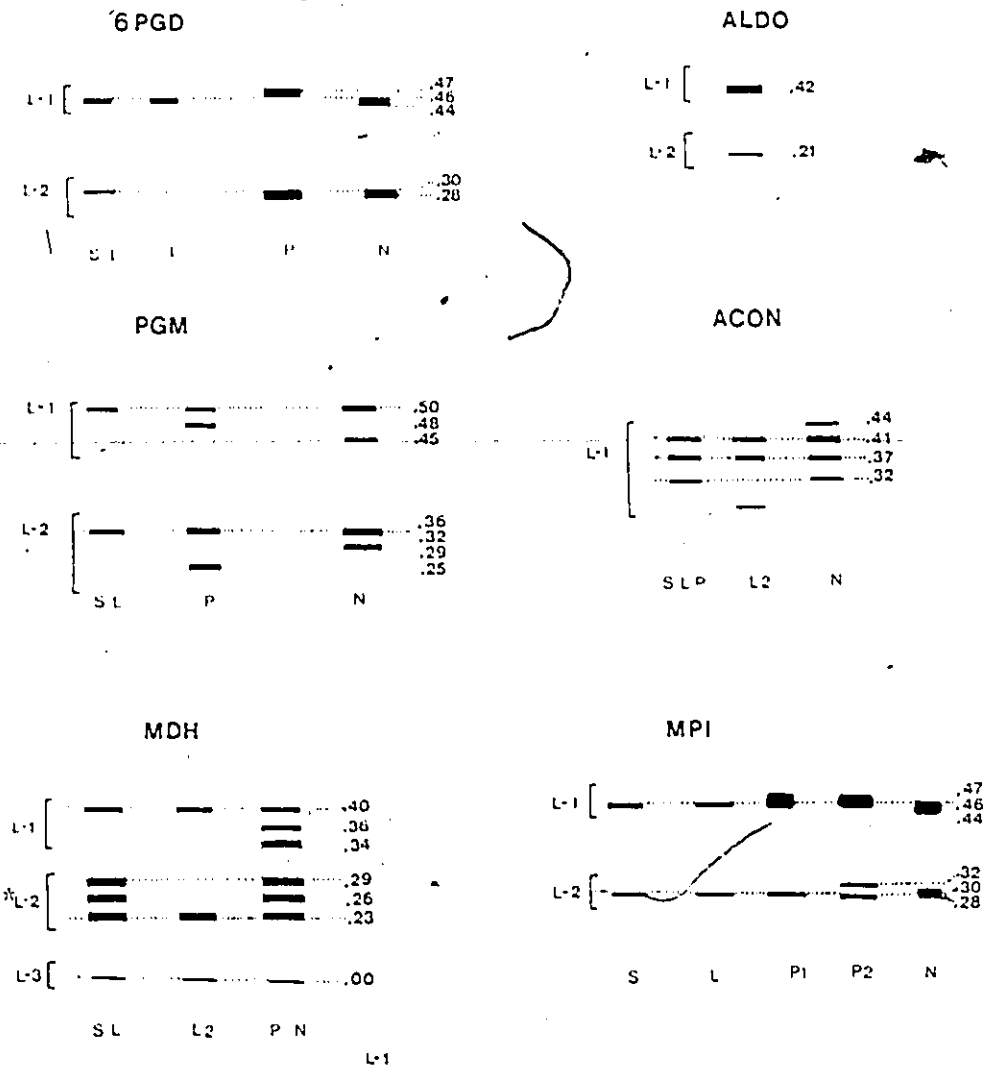

1
2
3
4
5
6
c

Figure 22: Banding patterns for 15 enzyme systems analyzed for all taxa.
Loci numbered L-1 (most anodal), L-2, L-3.

S= P. scabrum
L= P. lapathifolium
P= P. persicaria
N= P. pensylvanicum

R_f values given.


HK
L-1 .87
L-2 .82
GOT
L-1
L-2
SL P N
LAP
L-1 .67 .63
L-2 .28
L N S L P
GDH
L-1 .30 .28
S L P N
IDH
.47 .44 .41
G3PD
L-1 .36 .32 .27 .22 .18
S L P L N
TPI
L-1
*L-2 .78 .73 .70 .67 .61 .59 .54 .49
L (1 pop^n) S L L P N
PGI
L-1 .64 .63
L-2 .16 .14 .12 .10 .04 .00
S L L P N
SKDH
L-1 .45 .42 .40 .38 .35
L S L S L P1 P2 N



* not segregating at these loci, almost certainly fixed heterozygote from duplicated locus.

Four clearly resolved monomorphic loci were apparent for Hk-1, Idh, G3pd, Aldo-1, and Lap-1. A second locus was often resolved for Hk-2, Aldo-2 and Lap-2, however, these were less darkly stained and not always readable. When clear, they were monomorphic for a single allele, but were not included in further interpretations or calculations.

Hk-1, Aldo-1 and Idh were monomorphic for a single allele ($Hk\text{-}1^{87}$, $Aldo\text{-}1^{42}$ and Idh^{44}). Two shadow bands were also resolved at pH 5.7 for IDH at Rf .47 and Rf .42.

Lap-1, Skdh and G3pd were polymorphic, each locus having two or three alleles. $Lap\text{-}1^{87}$ was found in 90% of the North American populations (19/21) of P. lapathifolium, and $Lap\text{-}1^{63}$ was present in all European populations and collections and all P. scabrum populations (Figure 23a).

One allele of G3PD ($G3pd^{32}$) had a frequency of 1.00 in all North American populations, and one rarer allele found exclusively in several plants of two European collections from Helsinki, Finland ($G3pd^{27}$).

Three alleles were present for Skdh: two common ($Skdh^{45}$ and $Skdh^{40}$) and 1 rare ($Skdh^{42}$) (Figure 23b). The alleles $Skdh^{45}$ or $Skdh^{40}$ were found with a frequency of 1.00 in all but 7/45 populations of the diploid taxa in North America and Europe (Table 11). $Skdh^{42}$ was found in 3 populations of P. lapathifolium from North America. I have not been able to correlate the existence of these alleles with any other characteristics of the habitat, environment nor the plants themselves.

Figure 23. Starch gels stained for:

a: LAP
b: SKDH

L= P. lapathifolium var. lapathifolium
F= P. lapathifolium var. salicifolium
S= P. scabrum
P= P. persicaria
N= P. pensylvanicum
E= European P. lapathifolium.
For population codes see Appendix A.

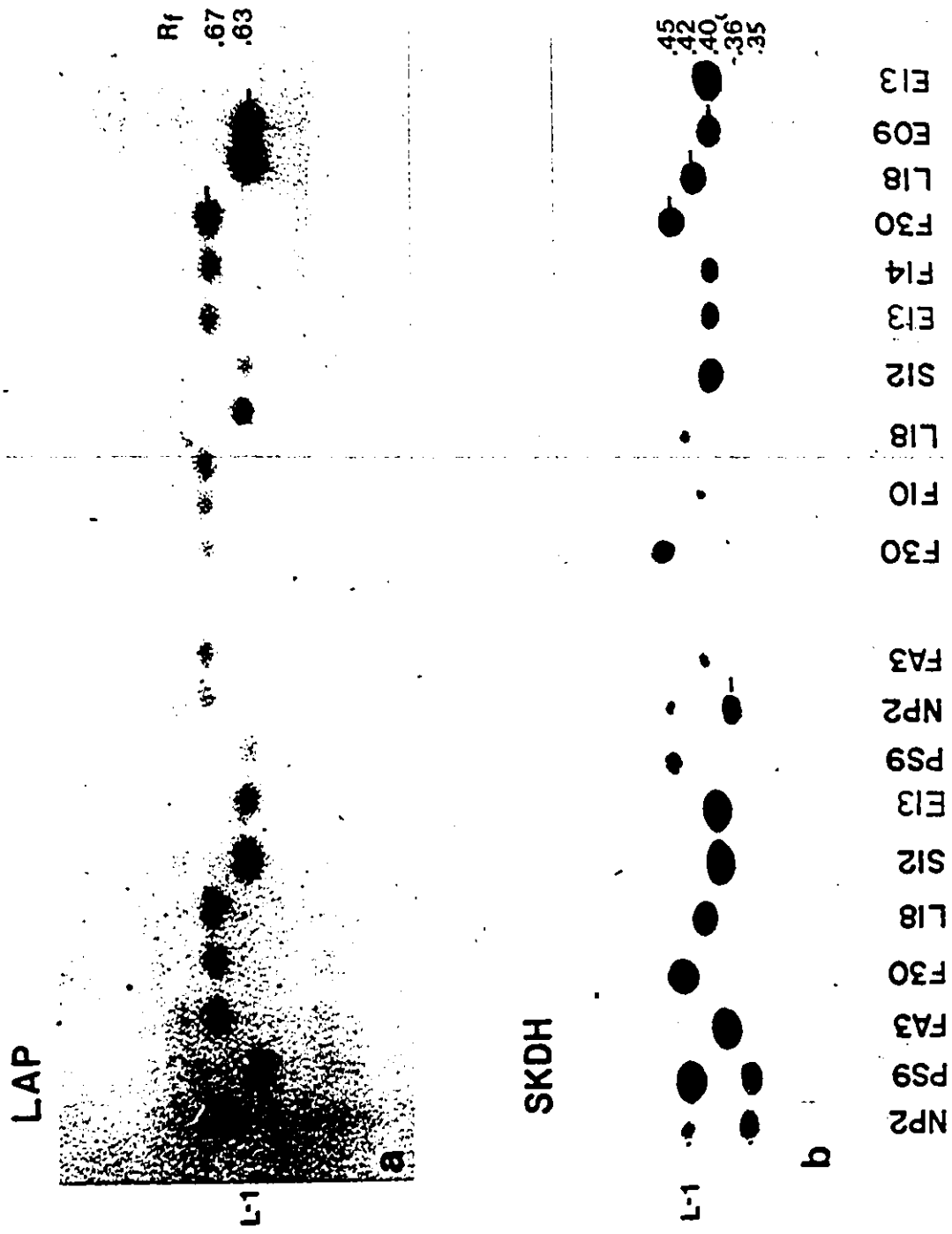

Rf
.67
.63
LAP
L-1
a
.45
.42
.40
.36
.35
SKDH
L-1
b
NP2
PS9
FA3
F30
LI8
SI2
EI3
PS9
NP2
FA3
F30
FI0
LI8
SI2
EI3
FI4
F30
LI8
EO9
EI3

Table 11: Frequencies of alleles of polymorphic loci in populations of Polygonum lapathifolium and P. scabrum. Refer to Appendix A for population codes. S = P. scabrum, L = P. lapathifolium var. lapathifolium, F= P. l. var. salicifolium, E = European P. l. ssp. tomentosum, N = P. l. ssp. lapathifolium.

	G3pd-.32	G3pd-.27	Pgi-2-.15	Pgi-2-.11	Skdh-.45	Skdh-.42	Skdh-.40	6Pgd-2-.30*
SC1	1.00		1.00		1.00			1.00
SC2	1.00		1.00		1.00			1.00
SC4	1.00		1.00		0.85		0.15	1.00
SC7	1.00		1.00				1.00	1.00
SC8	1.00		1.00				1.00	1.00
SC9	1.00		1.00				1.00	1.00
S10	1.00		1.00		1.00			1.00
S11	1.00		1.00				1.00	1.00
S12	1.00		1.00				1.00	1.00
S13	1.00		1.00				1.00	1.00
S14	1.00		1.00				1.00	1.00
S15	1.00		1.00		0.20		0.60	1.00
S19	1.00		1.00				1.00	1.00
L05	1.00		1.00				1.00	1.00
L12	1.00		1.00				1.00	1.00
L18	1.00		1.00			1.00		1.00
L20	1.00		1.00		1.00			1.00
L22	1.00		1.00			0.33	0.67	1.00
L23	1.00		1.00				1.00	1.00
L25	1.00		1.00				1.00	1.00
L27	1.00		1.00				1.00	1.00
L34	1.00		1.00		1.00			1.00
L36	1.00		1.00			1.00		1.00

Table 11 (cont.): Frequencies of alleles of polymorphic loci in populations of Polygonum lapathifolium and P. scabrum. Refer to Appendix A for population codes.

	G3pd·32	G3pd·27	Pgi-2·15	Pgi-2·11	Skdh·45	Skdh·42	Skdh·40	6Pgd-2·30*
FA3	1.00			1.00	0.13		0.87	1.00
F10	1.00			1.00	0.50		0.25	1.00
F13	1.00			1.00			1.00	1.00
F14	1.00			1.00			1.00	1.00
F15	1.00			1.00			1.00	1.00
F16	1.00			1.00			1.00	1.00
F17	1.00			1.00			1.00	1.00
F19	1.00			1.00	0.20		0.80	1.00
F26	1.00			1.00			1.00	1.00
F30	1.00			1.00	1.00			1.00
F31	1.00			1.00	1.00			1.00
F35	1.00			1.00			1.00	1.00
E091		1.00			1.00			
EO92	0.62	0.38		1.00			1.00	1.00
E11	1.00			1.00			1.00	1.00
E13	1.00			1.00			1.00	1.00
E14	1.00		0.23	0.77			1.00	1.00
N04	1.00			1.00			1.00	1.00
N08	1.00			1.00			1.00	1.00

Table 11 (cont.): Frequencies of alleles of polymorphic loci in populations of Polygonum lapathifolium and P. scabrum. Refer to Appendix A for population codes.

	Mdh-2·23	Mdh-2·29	Lap-1·67	Lap-1·63
SC1	1.00	1.00		1.00
SC2	1.00	1.00		1.00
SC4	1.00	1.00		1.00
SC7	1.00	1.00		1.00
SC8	1.00	1.00		1.00
SC9	1.00	1.00		1.00
S10	1.00	1.00		1.00
S11	1.00	1.00		1.00
S12	1.00	1.00		1.00
S13	1.00	1.00		1.00
S14	1.00	1.00		1.00
S15	1.00	1.00		1.00
S19	1.00	1.00		1.00
L05	1.00	1.00	1.00	
L12	1.00	1.00		1.00
L18	1.00	1.00	1.00	
L20	1.00	1.00	1.00	
L22	1.00	1.00	1.00	
L23	1.00	1.00	1.00	
L25	1.00	1.00	0.33	0.67
L27	1.00	1.00	1.00	
L34	1.00	1.00	1.00	
L36	1.00	1.00	1.00	

Table 11 (cont.): Frequencies of alleles of polymorphic loci in populations of Polygonum lapathifolium and P. scabrum. Refer to Appendix A for population codes.

	Mdh-2·23	Mdh-2·29	Lap-1·67	Lap-1·63
FA3	1.00	1.00	1.00	
F10	1.00	1.00	1.00	
F13	1.00	1.00	1.00	
F14	1.00	1.00	1.00	
F15	1.00	1.00	1.00	
F16	1.00	1.00	1.00	
F17	1.00	1.00	1.00	
F19	1.00	1.00	1.00	
F26	1.00	1.00	1.00	
F30	1.00	1.00	1.00	
F31	1.00	1.00	1.00	
F35	1.00	1.00	1.00	
E091	1.00		0.25	0.75
E092	1.00	1.00		1.00
E11	1.00	1.00		1.00
E13	1.00	1.00		1.00
E14	1.00	1.00		1.00
N04	1.00	1.00	1.00	
N08	1.00	1.00	1.00	

* alternate allele not present

One fixed multi-banded locus was coded for ACON and GDH in all populations.

The enzymes GOT (Figure 24b), MPI, 6PGD (Figure 24c), PGM, PGI, and TPI (Figure 24d) each possessed two loci; MDH possessed 3. In Got-1,-2, Mpi-1,-2, Pgi-1,-2, Pgm-1,-2, Pgi-1, Tpi-1 and Mdh-1,-3 each locus was monomorphic for a single allele.

In all populations, Tpi-2 was a fixed 3-banded phenotype ($Tpi\text{-}2^{67/49}$) (Figure 24d). In diploid plants, such a multiple enzyme phenotype may occur when a gene is duplicated, and the plant is fixed for alternate alleles. If it is a dimeric enzyme, the two alleles associate to form the intermediate heterodimer, the result being a three banded pattern. In one of the P. lapathifolium populations, the intensity was asymmetrical, with the most anodal band staining less intensely (Figure 25a). Mdh-2 also revealed a fixed multiple enzyme phenotype ($Mdh^{29/23}$) in all plants except two collections from Helsinki, Finland (the two collections possessing $G3pd^{27}$) (Figure 26).

Pgi-2 possessed two alleles: $Pgi\text{-}2^{14}$ was found only in one population from Reading, England. In Pgi-2 a double-banded pattern was found in all samples except P. pensylvanicum. This may be from a duplication of a gene and an unclear resolution of the fixed triple-banded pattern that is evident in P. pensylvanicum. A breakdown of the enzyme in the conditions used for analysis may also be the cause of this pattern.

The proportion of polymorphic loci for the species is 0.23, comparable to the same value for other inbreeding weedy plants

Figure 24: Starch gels of:

a: ACON

b: GOT

c: 6PGD

d: TPI

L= P. lapathifolium var. lapathifolium
F= P. lapathifolium var. salicifolium
S= P. scabrum
P= P. persicaria
N= P. pensylvanicum
E= European material
For population codes refer to Appendix A.

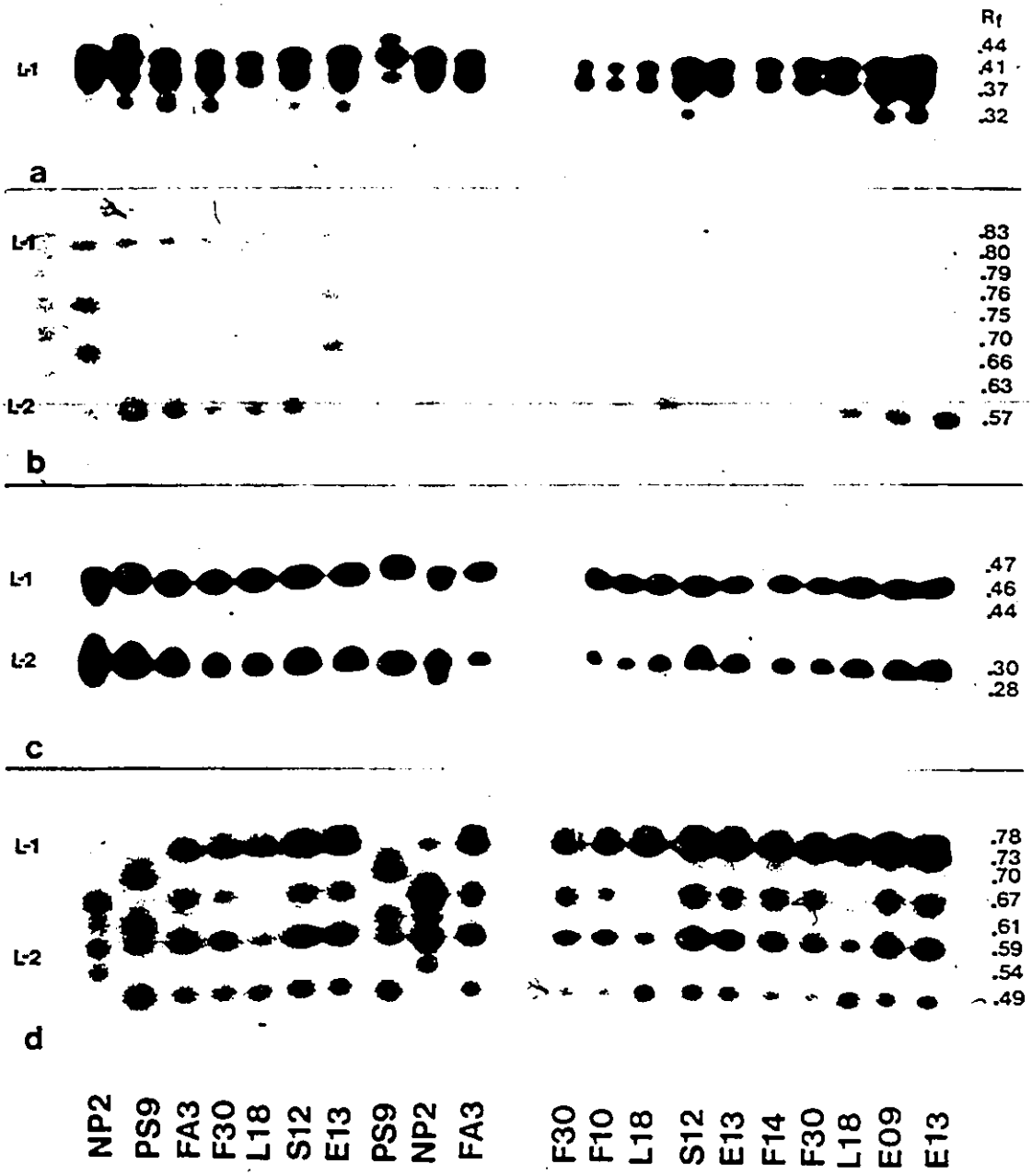

Rf
.44
.41
.37
.32
L-1
a
L-1
L-2
.83
.80
.79
.76
.75
.70
.66
.63
.57
b
L-1
L-2
.47
.46
.44
.30
.28
c
L-1
L-2
.78
.73
.70
.67
.61
.59
.54
.49
d
NP2
PS9
FA3
F30
L18
S12
E13
PS9
NP2
FA3
F30
F10
L18
S12
E13
F14
F30
L18
E09
E13

Figure 25: Starch gel stained for:

a: TPI

b: ACON

c: SKDH

d: LAP

Controlled amounts of buffer and plant material used to compare intensity of bands.

L= P. lapathifolium var. lapathifolium
S= P. scabrum
P= P. persicaria
For population codes refer to Appendix A.

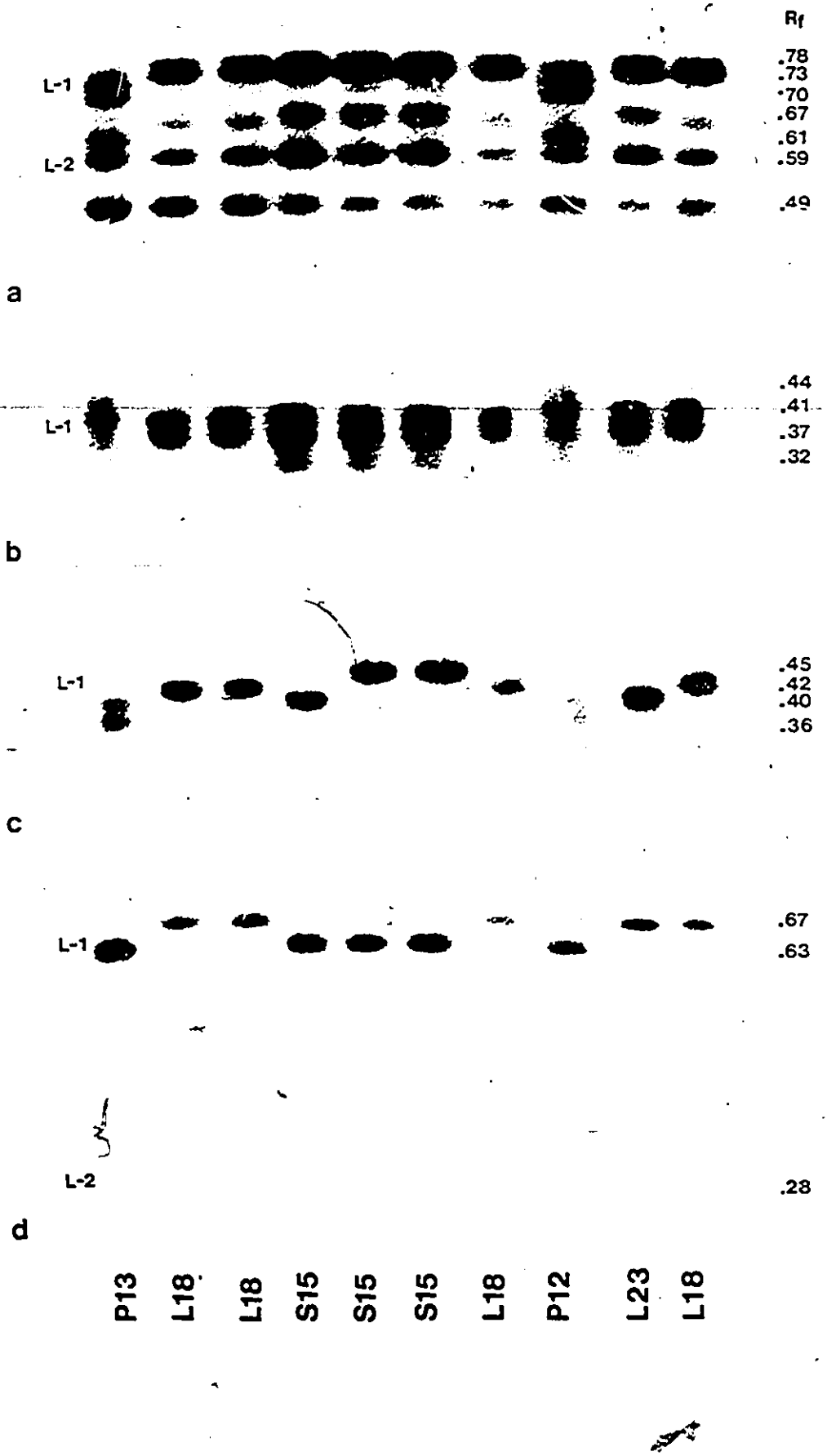
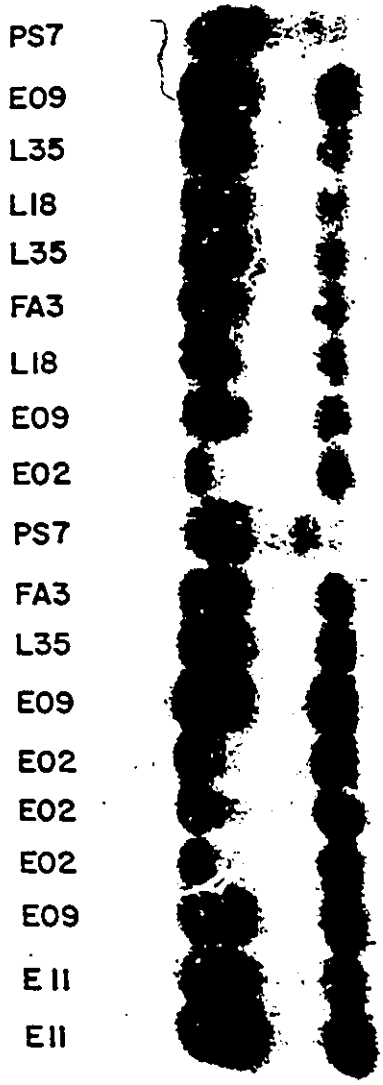
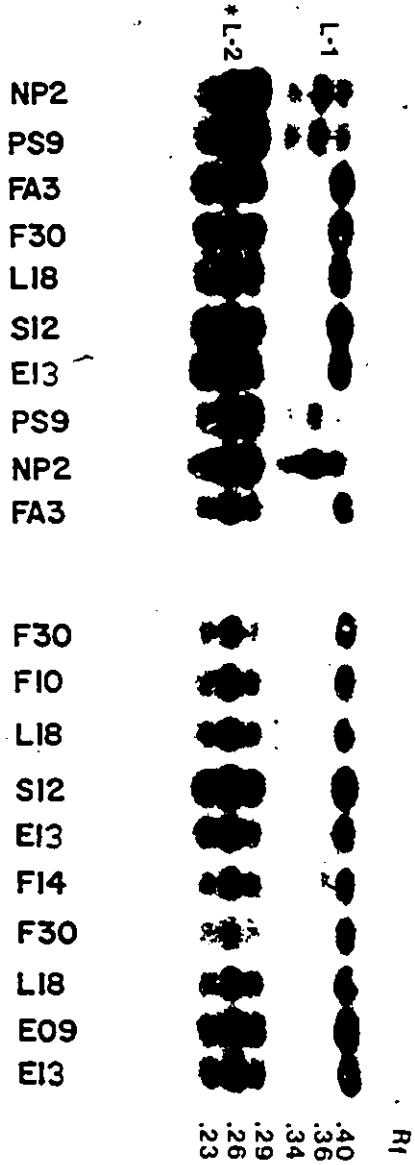

Rf
.78
.73
.70
.67
.61
.59
.49
L-1
L-2
a
.44
.41
.37
.32
L-1
b
.45
.42
.40
.36
L-1
c
.67
.63
L-1
L-2
.28
d
P13
L18
L18
S15
S15
S15
L18
P12
L23
L18

Figure 26: Starch gel stained for MDH.

L= P. lapathifolium var. lapathifolium
F= P. lapathifolium var. salicifolium
S= P. scabrum
P= P. persicaria
NP= P. pensylvanicum
E= European P. lapathifolium

* not segregating at L-2; almost certainly a fixed heterozygote from duplicated locus.


PS7
E09
L35
L18
L35
FA3
L18
E09
E02
PS7
FA3
L35
E09
E02
E02
E02
E09
E11
E11


MDH
*L-2
L-1
NP2
PS9
FA3
F30
L18
S12
E13
PS9
NP2
FA3
F30
F10
L18
S12
E13
F14
F30
L18
E09
E13
.23
.26
.29
.34
.36
.40
Rf

(Gottlieb, 1981).

3.8.1.2 P. persicaria and P. pensylvanicum (2n=44)

Duplication of the genome in these species was evident at loci Skdh, Got-1,-2, Gdh, Tpi-1,-2, Pgi-1, Pgm-1,-2, Mdh-1, Mpi-1,-2 and probably also in 6-Pgd-1,-2.

The banding pattern in the tetraploids was similar to that in the diploids for the loci Aldo-1 and Idh. In Hk-2, P. persicaria possessed the same pattern as that in the diploids but in P. pensylvanicum the second locus was never present. In LAP there was one locus in each of these species: Polygonum pensylvanicum possessed Lap^{67}, found in most North American P. lapathifolium, and P. persicaria possessed $Lap\text{-}1^{63}$, also found in all P. scabrum and European specimens. In G3PD, P. persicaria possessed the allele $G3pd^{32}$, also present in the diploids. Polygonum pensylvanicum had a fixed multi-banded pattern with the heaviest band at $G3pd^{32}$.

ACON and GDH revealed the same multi-banded pattern as in the diploids except: at the Acon locus, P. persicaria possessed an extra band at Rf .44; in P. pensylvanicum, an extra, more anodal band was present at Gdh^{29}.

In the enzyme SKDH, each of these species possessed two loci. $Skdh\text{-}1^{45}$ (found in the diploids) was present in both species. $Skdh\text{-}2^{35}$ was present in P. persicaria and $Skdh\text{-}1^{36}$ was found in P. pensylvanicum. One population of P. persicaria had a unique 2-banded pattern, with the tentative alleles $Skdh\text{-}1^{40}$ and $Skdh\text{-}2^{35}$.

In 6PGD, two loci were resolved. The bands were more darkly

stained than in the diploid plants. In P. persicaria, both are probably coded for by one allele: 6Pgd-1^{47} and 6Pgd-2^{30}. Polygonum pensylvanicum possessed 6Pgd-1^{44} and 6Pgd-2 appeared to possess at least two bands, however this locus was not clearly resolved (Figure 24). In PGM, both species possessed two different loci, each apparently duplicated and fixed for different alleles. Each species shared Pgm-1^{50} and Pgm-2^{32} of P. scabrum and P. lapathifolium. Polygonum persicaria also had Pgm-1^{48} and Pgm-2^{25}. Pgm-1^{45} and Pgm-2^{29} are present in P. pensylvanicum.

The two-locus pattern in MPI was also less clearly resolved in the tetraploid species than in the diploids. Mpi-1 was heavily stained and not clearly focussed in either species. It is, however, tentatively interpreted as Mpi-1^{47} in P. persicaria and Mpi-1^{44} in P. pensylvanicum. Both species appear to share Mpi-2^{28} with the diploid taxa. One low frequency pattern in P. persicaria revealed a second band at Rf .32.

Polygonum persicaria and P. pensylvanicum both possess two loci for MDH. At Mdh-2 the pattern is identical to that for P. lapathifolium and P. scabrum. Mdh-1^{40} is shared by all taxa, and in P. persicaria and P. pensylvanicum this locus is also a three-banded multiple enzyme pattern for Mdh-1$^{40/34}$. Two loci were found in PGI. Both species shared Pgi-2^{13} with P. lapathifolium and P. scabrum, and P. persicaria shared Pgi-1^{64} with them as well, and possessed an extra allele Pgi-1^{63}. Polygonum pensylvanicum possessed Pgi-1^{63}, and had a 3-banded fixed heterozygote Pgi-2$^{13/00}$. The three-banded multiple enzyme pattern in each of

these enzymes presumably corresponds to duplicated loci.

GOT and TPI have the most difficult patterns to interpret (Figure 25b, d). Alleles Got-1[80] and Got-2[52], present in P. lapathifolium and P. scabrum, are present and an additional two bands are present in P. persicaria at Rf .71 and .63. Polygonum pensylvanicum shares the Got-2[52] allele of P. lapathifolium and P. scabrum but has a unique pattern of six additional bands.

For TPI, the alleles Tpi-2[49] and Tpi-2[59] found in the diploid species are found in both tetraploids. The band Tpi-2[59] is also shared by all taxa. Polygonum pensylvanicum also has the allele at Rf .67 found in the diploids, and it is very heavily stained in this species. Polygonum persicaria and P. pensylvanicum have an extra band at Rf .61; P. persicaria two additional bands at Rf .73 and .70.

3.8.1.3 All taxa

Closely migrating pairs of bands (shadow bands) were found that do not seem to represent heterozygous phenotypes but instead be the result of either enzyme breakdown, or possibly a post-meiotic transformation. These were found in Pgm-2 in P. lapathifolium and P. scabrum, Mpi in one population of P. persicaria, Pgi-1 in P. persicaria and Pgi-2 in all taxa.

3.8.2 Similarity Indices

Twenty-eight alleles were identified in P. lapathifolium and P. scabrum (Figure 23). A total of twenty of these (71%) were found in each of P. persicaria (including Got-1[80], Pgi-1[64] and Lap-1[63]) and

P. pensylvanicum (including Tpi-1[73], and Lap-1[67]).

Twenty-four out of 53 bands (45%) were shared by P. persicaria and P. pensylvanicum.

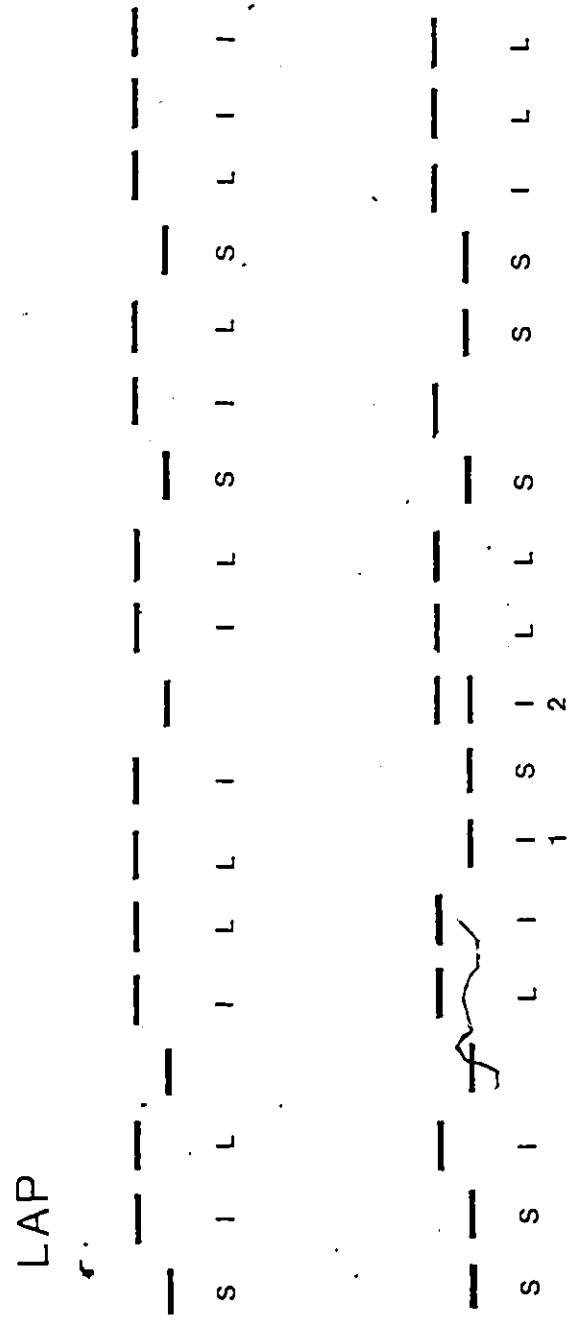
3.8.3. The North American P. lapathifolium complex

Very little isozyme variation was detected among P. scabrum and the two varieties of P. lapathifolium. Of the fifteen enzyme systems representing 23 loci, only two (LAP and SKDH) varied in the North American populations (Figure 23). SKDH was coded by a single locus with three alleles. Skdh[45] and Skdh[40] were not diagnostic for taxa, but most populations of P. scabrum and P. lapathifolium were homogeneous, possessing one or the other allele (Table 11). Skdh[42] was found in three populations of P. lapathifolium (Figure 25) which corresponded to the particular morphotype characterized primarily by a striking anthocyanin pattern in the stem (Figure 21) and in the inflorescence and glandularity on the peduncles and in the inflorescence. None of the European specimens possessed this allele.

Lap-1[67] was the only allele diagnostic for North American P. lapathifolium. Lap-1[67] was not found in any populations of P. scabrum, European collections, nor a collection of P. lapathifolium ssp. lapathifolium (as P. nodosum) from Japan. It was found in all North American populations of pure P. lapathifolium except one (Table 11).

In the mixed population (Dataset #3 in chapter 3) the plants identified as P. lapathifolium possessed Lap[67] and those identified as P. scabrum possessed Lap[63] (Figure 27). The intermediate plants

Figure 27: Electrophoretic pattern for enzyme LAP.
Samples as for Figure 16.


LAP
1
2

possessed Lap^{67} except for two cases: #1 possessed Lap^{63} (see Figure 16 for this plant's position in the PCA) and #2 possessed both bands: $Lap^{67/63}$.

3.8.4. Heterozygosity

Heterozygous individuals for both $Skdh^{45/40}$ and $Lap\text{-}1^{67/63}$ were found in a mixed population of P. lapathifolium and P. scabrum. These individuals were selfed and the progeny screened to examine segregation ratios and establish the genetic basis of the banding patterns. The results are shown in Table 12, and the banding pattern of the progeny of a selfed heterozygote ($Skdh^{45/40}$) is shown in Figure 28. Segregation of the alleles occurred in the progeny for both of these enzymes, and the X^2 test for goodness of fit reveals that the observed frequencies are the 1:2:1 expected ratio at the alpha=0.05 level.

3.8.5. Genotypes

With seventeen monomorphic loci (Hk-1, Got-1,-2, Gdh, Tpi-1,-2, Pgi-1, 6Pgd-1,-2, Aldo-1, Pgm-1,-2, Acon, Mdh-1,-3 Mpi-1,-2) and five polymorphic loci (Lap-1, Mdh-2, Skdh, G3pd and Pgi-2) coded for in the diploid taxa P. lapathifolium and P. scabrum , a total of 14 multilocus genotypes were present in these plants (Tables 13 & 14).

Genotypes 1 to 4 represent the specimens possessing $Lap\text{-}1^{67}$, and include almost all of the pure North American P. lapathifolium populations. These genotypes were not found in P. scabrum nor any European or Japanese plants.

Table 12: Results of progeny testing of two parent plants heterozygous for $Skdh^{.45/.40}$ and $Lap^{.67/.53}$.

Genotype	$Skdh^{.45/.45}$	$Skdh^{.45/.40}$	$Skdh^{.40/.40}$
Number of progeny	21	22	14

Genotype	$Lap^{.67/.67}$	$Lap^{.67/.53}$	$Lap^{.53/.53}$
Number of progeny	9	15	13

SKDH $X^2 = 4.79$ n.s. n=2, LAP $X^2 = 5.78$ n.s. n=2,
n.s.= non-significant at =0.05

Figure 28: Plant SL1-123X: Segregation of alleles in a heterozygous plant ($Skdh^{.45/.40}$). (Two plants of P. persicaria included, possessing $Skdh^{.36}$)

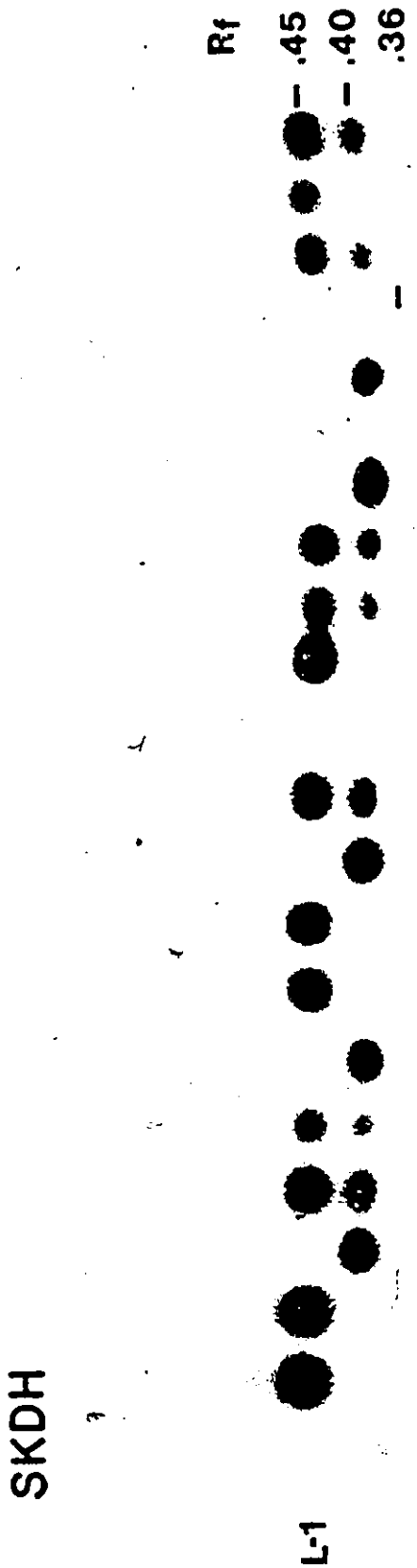

Rf
.45
.40
.36
SKDH
L-1

Table 13: Fourteen multi-locus genotypes in populations of Polygonum lapathifolium and P. scabrum from Europe and North America.

GENOTYPE	POLYMORPHIC LOCI				
1)	LAP-1·67	MDH-2·29/.23	SKDH·45	G3PD·32	PGI-2·12
2)	"	"	SKDH·45/.40	"	"
3)	"	"	SKDH·40	"	"
4)	"	"	SKDH·42	"	"
5)	LAP-1·67/.63	MDH-2·29/.23	SKDH·45/.40	"	"
6)	"	"	SKDH·40	"	"
7)	LAP-1·63	MDH-2·29/.23	SKDH·45	G3PD·32	"
8)	"	"	"	G3PD·24	"
9)	"	"	SKDH·45/.40	G3PD·32	"
10)	"	"	SKDH·40	"	PGI-2·12
11)	"	"	"	"	PGI-2·14
12)	"	MDH-2·23	SKDH·45	"	PGI-2·12
13)	"	"	SKDH·40	G3PD·32	"
14)	"	"	"	G3PD·24	"

Table 14: Population summary of electrophoretic genotypes. For population codes, refer to Appendix A.
S= P. scabrum, L= P. lapathifolium var. lapathifolium,
F= P. lapathifolium var. lapathifolium.
E= European populations.

GENOTYPE	TAXA and POPULATIONS
1	L20(3/3)1*, L27(5/5)2, F30(3/3)2, F31(1/1)2, F10(2/4)2, F19(1/5)1, SL1(2/2)2, SL5(13/13)1
2	LA3(2/8)3, F10(1/4)3
3	F19(4/5)1, L22(2/3)2, F10(1/4)3, F13(2/2)1, F14(3/3)2, F17(2/2)1, F35(5/5)2, L23(3/3)2, F26(2/2)1, LA3(6/8)3, L25(2/6)3, L34(10/10)1, LA5(2/2)1, F15(2/2)1, F16(1/1)1, SL1(3/23)1, SL4(3/4)1
4	L36(1/1)3, L18(8/8)5, L22(1/3)4
5	SL1(1/23), L25
6	SL1(1/23)3
7	SC1(3/3)2, SC2(3/3)1, SC4(5/7)1, S10(6/6)2, S15(2/5)4, E03(1/1)1, E09-1(1/5)5
8	E02-1(2/8)
9	SC4(5/9)2, S15(1/5)2, L25(1/6)3
10	E08(4/4), E09(4/5), E09-2(2/10), E11(3/3)3, E13(3/3)3, L12(2/2)1, E14(10/13)1, SC6(5/6)1, SC7(3/3)1, SC9(3/3)1, S12(4/4)2, S14(5/5)2, S15(2/5)2, S19(5/5)2, S13(4/4)1, SC8(1/1)1, S11(1/1)1, S16(1/1)1, E04(2/2)1, SL1(15/22)2, SL4(1/4)1
11	E14(3/13)1
12	E02-1(6/8)
13	E02-2(7/8)
14	E02-2(1/8)

*(n/m)p, n=number of parent plants with respective genotype;
m=total number of parent plants;
p=average number of progeny per parent plant with respective genotype.

Genotypes 5 and 6 are heterozygous for Lap-$1^{87/83}$ and represent specimens from the mixed population SL1.

Genotypes 6 to 13 are found in all P. scabrum, all European specimens and two populations of North American P. lapathifolium var. lapathifolium, and 3 of the mixed populations.

Among the "North American" genotypes (1-4) and the "European" genotypes (7-14) there is one genotype that occurs with a very high frequency (#3 and #10, respectively). The remainder of the genotypes occur at lower frequencies.

4. DISCUSSION

4.1. Cytology

The evidence presented here supports the majority of chromosome literature to date, with one exception. P. pensylvanicum was found to be tetraploid, with ca. 2n=44 chromosomes in pollen mother cells. This contradicts the report of Löve and Löve (1982) of 2n=22. The tetraploid ploidy level has been supported by Taylor-Lehman (in prep.) who has also found n=22 in six populations from the northeast United States. Moreover, the duplication of the genome evident in the isozyme patterns for 5 populations of P. pensylvanicum are consistent with all of these plants being tetraploid.

None of the specimens of the P. lapathifolium complex that I counted had a greater chromosome number than 2n=22. This agrees with 25 of the 28 previous counts of P. lapathifolium and P. scabrum. Therefore, from literature reports and chromosome counts performed here, it is possible to conclude the following:

P. persicaria and P. pensylvanicum are tetraploid (2n=44) and the taxa of the P. lapathifolium complex are diploid (2n=22). All of my evidence supports these ploidy levels, but the possibility of tetraploid levels within the P. lapathifolium complex cannot be ruled out in view of Löve and Löve's supposition in 1942 that plants they referred to as P. nodosum from Scania were autopolyploid with 2n=44 and therefore warrented specific status.

4.2. Morphology

The morphological study of primarily fruit characteristics revealed that taxa corresponding to P. lapathifolium and P. scabrum could generally be separated in north eastern North American wild and cultivated populations. It is evident, however, that the separation between P. lapathifolium and P. scabrum is not clear cut. This becomes particularly evident with the close examination of the mixed population. There is no morphological character that is unique for either of these taxa, and performing multivariate analyses on the OTU's did not succeed in clear separation. Within each group, dissections of the data set will result in smaller groups that differ in degree of characteristics (for example, the group spossessing the red anthocyanin pattern - see Section 3.7). Timson performed a study on fruit characters in 1963 comparing P. lapathifolium ssp. lapathifolium (as P. nodosum) and P. lapathifolium ssp. tomentosum (as P. tomentosum), in Europe. He, however, did not find significant differences between the two species and, therefore, suggested that they be considered subspecies of the species P. lapathifolium.

Although the leaf shape was quite variable within the complex, it is controlled by only one or two genes (Gottlieb, 1984). Metric traits representing such characters as fruit size, however, appear to be influenced by several loci (Gottlieb, 1984), typically ten or more genes (Lande, 1981). Therefore, the differences in fruit size found here are probably influenced by several genes and therefore merit consideration in classification.

4.3. Life History Characteristics

The difference in germination requirements between P. lapathifolium and P. scabrum is the strongest evidence for supporting separation into two taxa, and has not previously been reported. The difference between dormant and non-dormant seeds probably involve more than just a single gene. The genetics of coat imposed dormancy can be quite complex (Bewley and Black, 1985) because of the possible interactions between coat and embryo. This evidence supports the fruit size differences found. The correlation existing between these two traits suggests that taxonomic discrimination would be useful for these two groups, since in this case a morphological characteristic can quite reliably predict a biological trait, with both of these involving several genes. This need not be species recognition; examples are found in the literature in which a single species possesses different requirements for germination over its range, e.g. Avena fatua L. (Thurston, 1962), or else shows polymorphism within populations in this respect (e.g. Papaver in Harper and McNaughton (1960)). In fact, herbicide manufacturers breed strains free from dormancy, for example of P. lapathifolium, in order to overcome difficulties in testing of these plants (Harper, 1965). The exception to the pattern, P. lapathifolium in the mixed population germinating without stratification, supports the recognition of a single species, since it exemplifies genetic modifications that are perpetuated in discrete autogamous lines under appropriate conditions.

4.4. Flavonoids

In addition to cytological similarities reported to date by previous authors and this investigator, and in contrast to morphological, germination and flowering trends, a marked similarity in unhydrolyzed flavonoid constituents has been found for all plants (North American and European) in the P. lapathifolium complex. On the other hand, P. persicaria and P. pensylvanicum are distinguishable from members of the complex in respect to flavonoid evidence, supporting data from the other sources in this study.

4.5. Electrophoresis

4.5.1. Enzyme polymorphism and genetic variability in the P. lapathifolium complex.

It is evident from the results presented that selection has not been strong on this suite of enzymes in these taxa, and has been particularly limited since the establishment of populations in North America. Among the 15 enzymes representing 22 loci, only five loci showed variation. This low allozyme variability could be due to a limited introduction and founder effect, and/or a lack of selection or divergence after establishment. Since variation among European populations was also low, this rules out that the low levels of variation in North America are due exclusively to founder effects. It also supports the idea of a single species.

4.5.2. Locus Duplication

Duplication of gene loci in the diploid taxa was apparent for three enzymes, at loci Acon, Mdh-2, and Tpi-2, in the members of the P. lapathifolium complex. This was presumed because each revealed a three-banded true breeding phenotype. In diploid plants, this would occur when a gene was duplicated, and the plant is homozygous for a different allele at each of the two loci. For a dimeric enzyme, the isozymes coded by the different genes can associate to form the active heterodimer (Gottlieb, 1977). There was only one collection, from Helsinki, Finland, which contained some plants missing the allele Mdh-2^{29}. Previous evidence has indicated that duplications do not occur frequently in diploid species. However, Gottlieb (1977) states that it probably does occur more frequently than presumed, by multiple reciprocal translocations. His study of the structural gene PGI in Clarkia shows that duplication of this gene occurred during evolution of this genus and the duplicated segments were made homozygous by self-pollination.

As described in Section 3.7.1, two plant collections from Helsinki, Finland, have specimens that lack the Mdh-2^{29} allele. Mdh-2^{29} and an intermediate band do occur in other plants within these collections; therefore, the lack of Mdh-2^{29} is likely the result of either a simple mutation silencing this allele or a null allele being present at this gene location in these few plants lacking Mdh-2^{29}. Since the variation was found in only two collections from Europe, and no other characteristics were noted to separate these plants from the others in the same collection, this

variation was not considered taxonomically important within the complex. It would be worthwhile to survey other related species in the section to see if they possess any of these duplications. Such duplications have considerable phylogenetic importance above the species level, since organisms possessing the duplications inherited them from a common ancestor, thus belonging to a single monophyletic assemblage (Gottlieb, 1983).

4.5.3. Congruence of High Genetic Identity with Other Evidence.

Nei's (1972) genetic identity (I) calculations were not valid for this study because of the small population size investigated. However, with only two loci revealing variation in North America, "I" would be greater than 0.97, which is that indicated for inbreeding conspecific populations (Gottlieb, 1977; Crawford, 1983). Crawford (1983) stated that with inbreeding plants, the "species are either monomorphic at nearly every locus over their entire geographic ranges", or show "substantial interpopulational variation due to large differences in allelic frequencies at a number of loci". The plants in this complex conform to the former case.

Examples of disconcordance between allozyme and life-history characteristics have been given for colonizing species by Brown and Marshall (1981), Barrett and Richardson, (1985), Jensen et al (1979). Warwick et al. found very little allozyme divergence among populations of Setaria faberi L. (Warwick et al., 1987), Abutilon theophasti Medic. (Warwick and Black, 1986), and Sorghum halapense (L.) Pers. (Warwick et al., 1986) that showed high levels of

variation for life history and morphological traits. Warwick (1987) investigated several biotypes of cultivated and weedy strains of proso millet (Panicum miliaceum L.) and found that only two of the 11 enzymes showed variation among individuals. It was suggested that the divergence of the weedy strains was a recent occurrence and thus not reflected in the allozyme characters. In the present study, electrophoretic evidence does not promote the separate recognition of P. scabrum in North America. This supports the flavonoid chemistry evidence, where no flavonoids were found to distinguish P. scabrum from P. lapathifolium (present study). It also supports morphological evidence, in part, in that only a few of the fruit characters are significantly different between the two main groups recognized, and within these groups there is wide variation. It does not support the strong relationship of these fruit characters with life history patterns, both of which involve complex gene interaction. Therefore, the amount of disconcordance of data in P. lapathifolium is not very great, and the general pattern reveals a very closely related group of plants that have shown very low divergence since the occupation of their present day sites.

4.5.4. Electrophoretic Evidence for a North American component

One polymorphic locus in North American plants, (Lap-1) possesses an allele that appears to be diagnostic for North American P. lapathifolium (including our var. salicifolium and part of var. lapathifolium. One diagnostic allele is a very small amount of evidence for the recognition of a distinct North American group.

Nevertheless, electrophoretic evidence underestimates the amount of variation between plants, and the consistency of the appearance of Lap-1^{67} was striking. As opposed to the more southwestern distribution of herbarium specimens of the introduced P. scabrum, the far northern extension of herbarium specimen collections of P. lapathifolium supports this.

Interestingly, the native North American tetraploid P. pensylvanicum possesses Lap-1^{67}, whereas the European tetraploid P. persicaria possesses Lap-1^{63}. This provides more support to the existence of the native North American P. lapathifolium.

4.5.5. Heterozygosity

Individuals heterozygous for both alleles of SKDH or LAP were rare: In mixed populations (of P. scabrum and P. lapathifolium), only 2/127 progeny plants analyzed were heterozygous for SKDH, and only 1/159 analyzed were heterozygous for LAP. This lack of heterozygous individuals provides evidence for autogamy in this plant group. The heterozygous individuals provide evidence for compatibility between plants with genotypes 1-4 and 7-14 and support for not recognizing two species.

4.5.6. Polygonum persicaria and P. pensylvanicum

These two taxa were quite distinct from each other, and from the remaining taxa in this study.

Polygonum pensylvanicum L.

Kubetin and Schaal (1979) studied enzyme systems in six

populations of this species from the United States and found that polymorphism occurred extensively in three enzyme systems: Alkaline phosphatatse (ALP), phosphohexose isomerase (they abbreviated this as PGI) and 6-phosphoglucose isomerase (6PGD). In my study no polymorphism was encountered among the 33 individuals representing five populations from Ontario. This is a very small sample size, because P. pensylvanicum was included in comparison with the main study group, the P. lapathifolium complex, and was not investigated intensively in itself. 6PGD was the only enzyme included here that showed variation in the Kubetin-Schaal study. They reported only one isozyme for 6PGD, whereas two isozymes were found in my study. Gottlieb (1980) reports that in routine studies of this enzyme, two isozymes are found: one in the chloroplast and one in the cytoplasm. Kubetin and Schaal analyzed frozen leaf samples from mature plants. I found that as plants reached maturity it was more difficult to extract many enzymes, and bands were often missing from older samples, as opposed to patterns obtained from young seedlings. The freezing of samples also resulted in less enzyme activity, especially for the enzymes SKDH, 6PGD, IDH, LAP, HK, ACON, and ALDO. The different age and treatment of material studied, plus the possibility that different varieties were represented in their investigation makes it invalid to closely compare our results. However, the majority of enzymes in their study were indeed monomorphic for identical alleles in all six populations, in agreement with the level of variation found in the 4 populations in the present study.

Polygonum persicaria L.

Babbel and Wain (1977) noted that a high level of self-fertilization tends to reduce genetic variability within populations, which is supported by electrophoretic evidence revealed here. Both intra- and inter-population variation in P. persicaria was quite low in the 98 individuals representing 12 populations. Extensive sampling over the entire world range of this species is recommended before any conclusive statements can be made, especially in association with the reported chromosome numbers of $2n=40$ and $2n=22$.

4.5.7. Evolution of P. pensylvanicum and P. persicaria

Electrophoretic evidence is valuable in helping to elucidate genetic relatednesss of closely related plant groups, at the genus level and below (Gottlieb, 1981). Of relevance here are studies of autogamous and allogamous derivation of species in which electrophoretic patterns make it evident that the derivative allopolyploid (for example tetraploid) receives the genetic complement of both parents (i.e. the genome is duplicated). Since 74% of the alleles found in the P. lapathifolium complex were found in both P. pensylvanicum and P. persicaria presumably a different species was also one of the progenitors for each of these tetraploids. It is very probable that a member of this complex possessing $Lap\text{-}1^{67}$ was a parent of P. pensylvanicum which also possesses this allele. On the other hand, P. persicaria possesses $Lap\text{-}1^{63}$, which is present in all of the European specimens and P. scabrum, which provides evidence that this

tetraploid is introduced from a European origin. Since there are alleles found in the tetraploid taxa that are not found in the diploids, the polyploid event is probably not a very recent one.

5. General Discussion on the Polygonum lapathifolium complex

In the initial stages of this work, populations were assigned to three taxa: P. scabrum, P. lapathifolium var. lapathifolium and P. lapathifolium var. salicifolium; analyses revealed that the picture was somewhat more complicated than this. First of all, it was found that only two groups could be quite predictably distinguished. The significance levels for the fruit characters between groups of populations of North American P. lapathifolium and P. scabrum under similar cultivation conditions, the separation of these two in ordination, and the trends found in life history characteristics justifies a recognition of these two widely variable taxa. These taxa may be of aid in weed control or wildflower cultivation in that the biology of these plants can be quite reliably predicted. Nevertheless, phenotypic plasticity of habit, leaf and fruit characters is not unknown; these measurements varied significantly between parent and progeny groups in several cases. When in a vegetative state, these plants cannot be identified. Morphologically there is no one unique character distinguishing one taxon from another; the distinction is made on a combination of characters that vary by degree. Moreover, with the presence of morphological intermediates, cytological evidence for 2n=22 throughout the complex, flavonoid uniformity and low levels of genetic variability in

allozyme characters, I propose that the recognition of these taxa be at subspecies level. The first of these subspecies will be called ssp. lapathifolium which includes our P. lapathifolium and European ssp. lapathifolium, similar in all characters except one locus in isozyme data. The second group will be called ssp. tomentosum. This group corresponds to our P. scabrum and European ssp. tomentosum, similar in all characters.

Within these two groups, in this study, several smaller groups of populations differing in genotype were also found. These groups were not distinguished by discrete diagnostic morphological characters. The pattern discovered supports the idea of small adaptive mutations being perpetuated in autogamous lines.

This study does not support the recognition of var. salicifolium Sibth., as used in current North American floras. As mentioned in Section 1.3.1.1, the name was initially wrongly applied, as Sibthorp's original description does not correspond with the description in these floras. Although the distribution of small plants fitting the description of var. salicifolium have been collected further north than those of var. lapathifolium, these plants grow larger in greenhouse conditions.

Key for two subspecies of P. lapathifolium in North America

Plants must be fruiting. The combination of listed characters is necessary for reliable prediction of life history characteristics:
ssp. lapathifolium: achenes require stratification for germination,

average of over 10 weeks to flowering; ssp. tomentosum: some achenes show no dormancy, average of less than 10 weeks to flower.

Polygonum lapathifolium L.

Achene 1.5-2.2 mm long, 1-2 mm wide. Inflorescence a long, narrow, drooping raceme. Perianth closed and often beaked over the inflorescence. Stem very often with red anthocyanin in ring under nodes and/or in spots on internodes. Little chlorophyll in fruiting perianths, which are often light to deep pink.–

ssp. lapathifolium

Achene 2.5-3.5 mm long, nearly as wide. Inflorescence relatively stout and rarely drooping. Perianth not closed over achene, rich in chlorophyll, lacking pink anthocyanin. Never red anthocyanin in ring under nodes or in spots on internodes. Very glandular peduncle and cilia ca. 1 mm long (just visible to the naked eye) on ochreae.–

ssp. tomentosum (Schrank) Danser.

6. Conclusions

The overall conclusion is that classification within P. lapathifolium (incl. P. scabrum) is complex. Characteristics that promote the success of this species as a weed, such as phenotypic plasticity of vegetative characters and a predominantly autogamous reproductive strategy, contribute to this complexity. In answer to the four original questions posed:

i. Evidence for native North American P. lapathifolium is not

strong. It consists of one allele of a polymorphic locus. In addition, the distribution of ssp. lapathifolium extends further north than that of the entirely introduced ssp. tomentosum. No diagnostic field characteristic exists, however, for distinguishing these plants from European members of the species.

ii Two widely variable subspecies should be recognized: ssp. tomentosum (= P. scabrum) and ssp. lapathifolium (=var. lapathifolium and var. salicifolium). Variety salicifolium is not recognized as a valid taxon as used in North American floras.

iii. Electrophoretic patterns support reports of P. lapathifolium as being diploid and P. persicaria and P. pensylvanicum as being tetraploid.

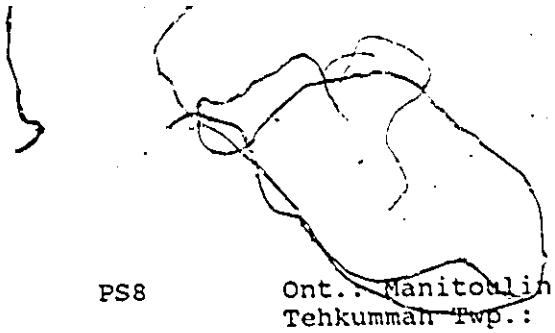
iv. Polygonum persicaria and P. pensylvanicum show allopolyploid derivation, with P. lapathifolium as one parent.

APPENDIX A: WILD POPULATIONS AND SEED COLLECTIONS 1984/85

TAXON	POPN#	LOCATION
lapathifolium var. lapathifolium	LA2	Ont.: Ottawa-Carleton Regional Municipality (RM) Vars, 1984
	LA3	New York: Ithaca, 1984 ambiguous identity; referred to as FA3 in electrophoretic results
	LA4	Ont.: Ottawa-Carleton RM. Ottawa, 1984
	LA5	Colorado: Larimer Co Fort Collins, 1984
	LA6	Ontario: Haldimand-Regional RM Rock Pt., 1984
	L12	Ont.: Manitoulin Dist.: Tehkummah Twp., 1985
	L18	Que.: Rouville Co.: Rougemont, 1985
	L20	Ont.: Prescott Co.: Longeuil Twp., 1985
	L21	Que.: D'Argenteuil Co.: Grenville Twp., 1985
	L22	Que.: D'Argenteuil Co.: Grenville Twp., 1985
	L23	Ont.: Ottawa-Carleton RM Vars, 1985
	L24	Ont.: Thunder Bay Dist.: Squaw Bay, 1985
	L25	Ont.: Thunder Bay Dist.: O'Connor Twp.: 1985
	L27	Ont.: Sudbury Dist.:

		W of North Bay, 1985
	L29	Ont.: Ottawa-Carleton RM Orleans, 1985
	L34	Ont.: Frontenac Co.: Kingston Twp.: Cateraqui, 1985
	L37	Ont.: Ottawa-Carleton RM 1984
(European) ssp. lapathifolium	N04	Portugal: Oeiras
	N05	France: Besancon
	N08	France: Nantes
lapathifolium var. salicifolium	F10	Ont.: Haliburton Co.: 1985 Harburn Twp.: Ross's L
	F11	"
	F13	Ont.: Manitoulin Dist.: 1985 Mills Twp.: Shrigley Bay
	F14	Ont.: Cochrane Dist.: Deloro Twp.: Delnite 1985
	F15	Ont.: Nipissing Dist.: Pentland Twp.: Kiosk 1985
	F16	Ont.: Renfrew Co.: N Algoma Twp.: Golden L 1985
	F17	Que.: Wolfe Co.: Price Twp.: Lac St. Francois 1985
	F19	Que.: Rouville Co.: Mont St. Hilaire 1985
	F26	Ont.: Thunder Bay Dist. Dorion Twp.: Wolf R 1985
	F30	Ont.: Kenora Dist.: Carroll L. 1985

	F31	Ont.: Kenora Dist.: Carroll L. 1985
	F35	Ont.: Thunder Bay Dist.: Brule Bay 1985
	J01	Japan, 1986
pensylvanicum	NP1	Ont.: Ottawa-Carleton RM Ottawa 1984
	NP2	Ont.: Ottawa-Carleton RM Orleans, 1984
	NP3	Ont.: Ottawa-Carleton RM, 1984
	NP4	Ont.: Haldimand-Norfolk RM: Rock Pt., 1984
	NP5	New York: Ithaca 1984
	NP6	Ont.: Manitoulin Dist.: Howland Twp.: Little Current 1985
	NP7	Ont.: Ottawa-Carleton RM 1985
persicaria	PS1	Ontario: Prescott Co. 1984 S. Plantagenet Twp. St. Isidore de Prescott
	PS2	Ont.: Lanark Co.: Beckwith Twp. 1984
	PS3	New York: Ithaca 1984
	PS4	Ont.: Durham Co.: Darlington Twp. 1984
	PS5	Ont.: Haldimand-Norfolk RM Jarvis. 1984
	PS6	Ont.: Haldimand-Norfolk RM Townsend 1984
	PS7	Austria: Neider Osterreich Melk 1984?



	PS8	Ont.: Manitoulin Dist.: Tehkummah Twp.: Leason Bay 1985
	PS9	Ont.: Manitoulin Dist.: Howland Twp: Little Current 1985
	PS10	Ont.: Nipissing Dist: Pentland Twp.: Kiosk, 1985
	PS12	England: Berkshire: Reading, 1985
	PS13	England: Berkshire: Reading, 1985
	P14	Ont.: Ottawa-Carleton RM, 1984 Vars
	E05	France: Besancon
	E06	France: Caen
	E12	Portugal: Lisbon
scabrum	SC1	Ont.: Ottawa-Carleton RM Ottawa, 1984
	SC2	Ont.: Ottawa-Carleton RM: Ottawa 1984
	SC4	Ont.: Lanark Co.: Beckwith Mun.: Mississippi L 1984
	SC5	Ont.: Ottawa-Carleton RM: Ottawa 1984
	SC7	Ont.: Prescott Co.: Caledonia Twp.: Alfred 1985
	SC8	Ont.: Timiskaming Dist. Dymond Twp.: New Liskard 1985
	SC9	Ont.: Nipissing Dist. Pentland Twp.: Kiosk 1985
	S10	Ont.: Renfrew Co.: Clara Twp.: Deux Rivieres 1985

	S11	Que.: Rouville Co.: St. Paul d'Abbotsford 1985
	S12	Que.: Rouville Co.: St. Paul d'Abbotsford 1985
	S13	Que.: Megantic Co.: Ireland Twp.: Black L 1985
	S14	Que.: Megantic Co.: Ireland Twp.: Black L 1985
	S15	Que.: Frontenac Co.: Lambton Twp.: Riviere aux bluets 1985
	S16	Que.: Moncalm Co.: Pau Twp.: Riviere d'Argent 1985
	S17	Ont.: Ottawa-Carleton RM Vars 1984
	S19	Ont.: Thunder Bay Dist.: Port Arthur 1985
	E01	Finland: Oulu
referred to as ssp. tomentosum)	E02-1	Finland: Helsinki
	E02-2	Finland: Helsinki
	E03	Norway: Oslo
	E09-1	W. Germany: Oldenburg
	E09-2	W. Germany: Oldenburg
	E11	E. Germany: Halle
	E12	Portugal: Lisboa
	E13	Austria: Nieder Osterreich
	E14	England: Berkshire: Reading

mixed
(P.lapathifolium
& P. scabrum)

SL1	Ont.: Prescott Co.: S. Plantagenet Twp.: St. Isidore de Prescott
(SM)	plants collected as P. scabrum in 1985
(LM)	plants collected as P. lapathifolium in 1985
(IM)	plants collected as intermediates in 1985
SL2	Ont.: Ottawa
SL3	Ont.: Lanark Co.: Ramsay Twp.: Almonte
SL4	Ont.: Manitoulin Dist.: Howland Twp.:
SL5	Ont.: Hamilton-Wentworth RM Toll Gates, Hamilton

APPENDIX B: WILD POPULATIONS AND SEED COLLECTIONS 1984/85
Specimens used for analyses.

TAXON	POPN	MORPH.	ISOZ.	FLAV.	CHROM. COUNTS
lapathifolium var. lapathifolium	LA3	4	8	5	
	LA5		2		1
	LA6				
	L12	5	2	1	
	L18	5	8	2	
	L20	5	3		1
	L22	5	3		
	L23	5	3		
	L25	5	6	1	
	L27	5	5		
	L34	5	10		
lapathifolium var. salicifolium	F10	6	4	1	
	F11	3			
	F13	2	2		
	F14	3	3		
	F15	5	2	1	
	F16	5	1		
	F17	5	2		
	F19	5			
	F26	3	2		

	F30	3	3		
	F31	1	1		
	F35	5	5	2	
European P. lapathifolium	E01				
	E02-1		8		
	E02-2		8		
	E03	5	1		
	E04	5	2		
	E05	2			
	E08	5			
	E09-1	5	6	1	
	E09-2		1		
	E11	5	3		
	E13	3	3		
	E14	5	13		
ssp. mesomorphum	E09-3		5		
ssp. nodosum	E08	5	3	1	
mixed (P.lapathifolium & P. scabrum)	SL1		26	2	1
	SL2		2	1	
	SL3		6		1
	SL4		12	3	
	SL5		13		

pensylvanicum	NP1	10	6	1	6
	NP2				
	NP3	3	2		
	NP4	3	1		
	NP5	2	2	1	
	NP6				
	NP7		1		
persicaria	PS1	10	12		
	PS2	10	1		
	PS3	10	1	1	
	PS4	10			
	PS5	10	4		
	PS6	10	6		
	PS7		3		
	PS8		1		
	PS9		3		
	PS10		4	1	
	PS12		2		
	PS13		3		
	P14				
	EO5		2		

scabrum	SC1	4	5		
	SC2	3	3		
	SC4	7	9	4	
	SC7	5	3	1	
	SC8		1		
	SC9	3	3	1	
	S10	5	6	3	
	S11	3	1		
	S12	6	4	3	
	S13	5	4	1	3
	S14	2	5		
	S15	5	5		
	S16	5	2	1	
	S17	4			
	S19	5	5		
	SM	30	9		
	LM	30	11		
	IM	29	12		

Appendix C. Data on P. lapathifolium lectotype specimen, designated by Timson, 1963):
Hort Cliff. 42. (J. McNeill pers. comm. in response to checklist prepared by L.L. Consaul).

Persicaria florum staminibus quinis, stylo bifido, corolla regular, aequalibus.
Persicaria major Lapathi foliis, calyce floris purpureo. Tournef. 510.

1) Length of achene 2.3-2.5mm
Width of achene 1.55-1.8mm

2) Perianth beaked around achene (top of perianth & top of styles about equal.

3) Glandularity none to sparse on calyces & ochreolae; moderately dense on peduncles and inflorescence branches.

4) Leaf shape.

5) Colour: may have had pink perianth and stem.

6) Ochrea: uppermost on stem below inflorescence has cilia 0.15-0.45mm long. most near 0.15mm. In lower inflorescence region: 0.15-0.2mm

7) Leaves are sparsely hairy.

Appendix D: Germination of seed from North American populations of the P. lapathifolium complex. Percentage = (%); positive 2,3,5 tetrazolium chloride test= [t].

POPULATION	WITHOUT STRATIFICATION (%)[t]	WITH STRATIFICATION (%)
F10	0/25 (0)	38/105 (36)
F13	0/25 (0)	6/13 (46)
F14		6/8 (75)
F15	0/10 (0)	6/75 (8)
F16	0/12 (0)	21/106 (20)
F19	0/50 (0) 0/50 [progeny]	68/132 (52)
F30	0/30 (0) 0/25 [progeny]	18/36 (50)
F35	0/55 (0) 0/75 [progeny]	72/82 (88)
LA3	0/25 [progeny]	31/97 (32)
LA5	0/10 (0)	31/38 (82)
LA6	0/20 (0)	4/76 (5)
L12	0/13 (0)	77/160 (48)
L17	0/43 (0)	3/72 (5)
L18	0/80 (0)	151/613 (24)
L20	0/45 (0)	39/72 (54)
L22	0/25 (0)	17/93 (18)
L23	0/25 (0)	16/78 (21)
L25	0/50 (0)	76/121 (63)
L27	0/50 (0)	76/120 (76)
SC1	35/60 (58)	69/114 (61)

Appendix D (cont.):

SC2	30/40 (75)	43/65 (66)
SC4	227/412 (55)	96/207 (46)
SC7	14/85 (16)	3/15 (20)
SC8	6/10 (60)	6/10 (60)
SC9	38/68 (56)	12/20 (6)
S10	148/244 (61)	15/15 (100)
S11	0/50 (0) 22/75 (30)	12/37 (32)
S12	13/85 (15)	31/58 (53)
S13	258/310 (83)	5/10 (50)
S14	68/110 (62)	
S15	33/60 (55)	119/125 (95)
S16	7/95 (7)	7/18 (39)
S19	144/230 (63)	1/5 (20)
SL1 (var. lapath.)	322/640 (50)	208/566(37)[3]
SL1 (scabrum)	635/694 (92)	588/738(80)[0]
SL1 (intermed.)	249/859 (29)	187/955(20)[19]

APPENDIX E: STAIN AND EXTRACTION BUFFER RECIPES

EXTRACTION BUFFER:

H_2O	90ml
Tris-Sigma (7-9)	1.20g
EDTA	38mg
KCl	74mg
0.1M $MgCl_2$	10ml
to pH 7.5 with HCl.	
Triton-X100	0.4ml
2-mercaptoethanol	10 ul/10ml buffer.

Leucine aminopeptidase

Boric acid soak:		0.25M	
LAP A		NaOH	8g/l
		Maleic anhydride	17.3g/l
LAP B		NaOH	12.8g/l

Stain:	LAP A	50ml	
	LAP B	10ml	
	H_2O	40ml	
L-leucyl-b-naphthylamide HCl			70mg
Black K salt			30mg

Aconitase

0.1M Tris-HCl (unadj.)		75ml
cis-aconitic anhydride		90mg
NADP		10mg
to pH 8.0 with HCl		
IDH	120ul	
0.1M $MgCl_2$	4.0ml	
MTT	0.75ml	
PMS	pinch	

Aldolase

0.1M Tris-HCl pH8.0	75ml
Fructose 1,6 diphosphate	190mg
NAD	30mg
Na_2 arsenate	225mg
Gly-3PDH	10.0ul
MTT	0.75ml
PMS	pinch

Glutamate dehydrogenase

0.1M Tris-HCl pH8.5	75ml
Glutamic acid monosodium salt	4.5g
NAD	38mg
MTT	75ml
PMS	pinch

Glyceraldehyde-3 phosphate dehydrogenase

0.1M Tris-HCl pH 7.7	75ml
Fructose 1,6 diphosphate	130mg
Aldolase	50ul
Incubate @ 37C for 30 min, then pour into:	
NADP	8mg
Na_2 arsenate	60mg
EDTA	90mg
0.1M $MgCl_2$	4mL
MTT	.75ml
PMS	pinch

Glutamate oxaloacetate transaminase

substrate	50ml
H_2O	50ml
Fast Blue BB	100mg
substrate, pH 7.5:	
H_2O	50ml
-ketoglutaric acid	36.5mg
L-aspartic acid	133.1mg
PVP-40	0.5g
EDTA	50mg
Na_2HPO_4	1.42g

Hexokinase

0.1M Tris-HCl pH 8.0	75ml
Glucose	275mg
ATP	45mg
NADP	10mg
G6PDH (40units/ml)	1.0ml
0.1M $MgCl_2$	4.0ml
MTT	0.75ml
PMS	pinch

Isocitrate dehydrogenase

0.1M Tris-HCl pH 8.0	75ml
Isocitrate	150mg
NADP	8mg
0.1M $MgCl_2$	4.0ml
MTT	0.75ml
PMS	pinch

Malate dehydrogenase

0.1M Tris-HCl pH 9.1	75ml
Malic acid	150mg
NAD	30mg
MTT	1.5mg
PMS	pinch

Mannose phosphate isomerase

0.1M Tris-HCl pH 8.0	75ml
PGI (yeast)	30 units
G6PDH	15 units
0.1M $MgCl_2$	7.5 ml
NADP	8mg
Mannose-6-phosphate	23mg
MTT	0.75ml
PMS	pinch

Phosphoglucose isomerase

0.1M Tris-HCl pH 8.3	75mL
Fructose-6-phosphate	35mg
NADP	4mg
G6PDH (40units/ml)	0.75mL
0.1M $MgCl_2$	4mL
MTT	0.75mL
PMS	pinch

Phosphoglucose mutase

0.1M Tris-HCl pH 8.0	75mL
Glucose-1-phosphate	57mg
NADP	4mg
G6PDH (40units/ml)	0.75mL
0.1M $MgCl_2$	40mL
MTT	0.75mL

PMS	pinch

Shikimic acid dehydrogenase

0.1M Tris-HCl pH 8.5	75mL
NADP	38mg
MTT	0.75mL
PMS	pinch

Triose phosphate isomerase

0.1M Tris-HCl pH 8.0	75ml
DHAP	10mg
EDTA	30mg
NAD	23mg
Na_2 arsenate	345mg
Gly-3PDH	200units
MTT	0.75ml
PMS	pinch

6-phosphogluconate dehydrogenase

0.1M Tris-HCl pH 8.0	75ml
6-phosphogluconate (Ba salt)	45mg
NADP	4mg
0.1M $MgCl_2$	4mL
MTT	0.75ml
PMS	pinch

APPENDIX F: Multiple comparisons of character state means using the Tukey-Kramer option of Duncan's multiple range test.

Data Set #1:

LA= P. lapathifolium, SC= P. scabrum, PS= P. persicaria

Character	Taxon Comparison	Significance: ** = significant at alpha=0.01
1	LA-SC	
	LA-PS	**
	SC-PS	**
2	LA-SC	**
	LA-PS	
	SC-PS	**
4	LA-SC	**
	LA-PS	
	SC-PS	**
5	LA-SC	**
	LA-PS	
	SC-PS	
6	LA-SC	**
	LA-PS	
	SC-PS	**
7	LA-SC	**
	LA-PS	
	SC-PS	**

APPENDIX F (CONTINUED):

Data Set #2: (a=with SC1, S14, SC9, SC6, SC7; b= without)

E= European P. lapathifolium ssp. tomentosum + 2 ssp. lapathifolium
L= North American P. lapathifolium var. lapathifolium
Y= as L, but cultivated
F= P. lapathifolium var. salicifolium
Z= as F, but cultivated
S= P. scabrum
X= as S, but cultivated

Character	Group Comparison	Significance: ** = significant at alpha=0.01	
		a	b
1	E-X		**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z	**	**
	X-Y	**	
	X-F	**	
	X-L	**	
	X-S	**	
	X-Z	**	
	Y-F		
	Y-L		
	Y-S		
	Y-Z		
	F-L		
	F-S		
	F-Z		
	L-S		
	L-Z		
	S-Z		
2	E-X	**	
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S		
	E-Z	**	**
	X-Y	**	**
	X-F	**	**
	X-L	**	
	X-S	**	
	X-Z	**	**

APPENDIX F (CONTINUED):

Character	Group Comparison	Significance: ** = significant at alpha=0.01	
	Y-F		
	Y-L		
	Y-S	**	**
	Y-Z		
	F-L		
	F-S	**	**
	F-Z		
	L-S	**	**
	L-Z		
	S-Z	**	**
4	E-X		**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z	**	**
	X-Y	**	**
	X-F	**	**
	X-L	**	**
	X-S	**	
	X-Z	**	**
	Y-F	**	**
	Y-L	**	**
	Y-S	**	**
	Y-Z		
	F-L		
	F-S	**	**
	F-Z		**
	L-S	**	**
	L-Z	**	**
	S-Z	**	**
5	E-X		**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z	**	**
	X-Y	**	**
	X-F	**	**
	X-L	**	**
	X-S	**	
	X-Z	**	**
	Y-F	**	**

APPENDIX F (CONTINUED):

Data Set #2

Character	Group Comparison	Significance: ** = significant at alpha=0.01 a	b
	Y-L		
	Y-S	**	**
	Y-Z	**	**
	F-L	**	**
	F-S	**	**
	F-Z		
	L-S	**	**
	L-Z	**	**
	S-Z	**	**
6	E-X		
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z	**	**
	X-Y	**	**
	X-F	**	**
	X-L	**	**
	X-S	**	
	X-Z	**	**
	Y-F		
	Y-L		
	Y-S	**	**
	Y-Z		
	F-L		
	F-S	**	**
	F-Z		
	L-S	**	**
	L-Z		
	S-Z	**	**
7	E-X	**	**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z	**	**
	X-Y	**	**
	X-F	**	**
	X-L	**	**
	X-S	**	
	X-Z	**	**

APPENDIX F (CONTINUED):

Data Set #2

Character	Group Comparison	Significance: ** = significant at alpha=0.01	
		a	b
	Y-F	**	
	Y-L		
	Y-S	**	**
	Y-Z		
	F-L		
	F-S	**	**
	F-Z		
	L-S	**	**
	L-Z		
	S-Z	**	**
8	E-X		
	E-Y		
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z		
	X-Y		
	X-F	**	**
	X-L	**	**
	X-S		
	X-Z		
	Y-F	**	**
	Y-L		
	Y-S		
	Y-Z		
	F-L		
	F-S		
	F-Z	**	**
	L-S		
	L-Z	**	**
	S-Z		
9	E-X	**	**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z		
	X-Y	**	**
	X-F	**	**
	X-L	**	**
	X-S		

APPENDIX F (CONTINUED):

Data Set #2

Character	Group Comparison	Significance: ** = significant at alpha=0.01	
		a	b
	X-Z		
	Y-F		
	Y-L	**	**
	Y-S	**	**
	Y-Z	**	**
	F-L	**	**
	F-S	**	**
	F-Z	**	**
	L-S	**	**
	L-Z	**	**
	S-Z		
10	E-X	**	**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S		
	E-Z		
	X-Y	**	**
	X-F	**	**
	X-L	**	**
	X-S		
	X-Z		
	Y-F		
	Y-L	**	**
	Y-S	**	**
	Y-Z	**	**
	F-L	**	**
	F-S	**	**
	F-Z	**	**
	L-S	**	**
	L-Z	**	**
	S-Z		
11	E-X	**	**
	E-Y		
	E-F		
	E-L	**	**
	E-S		
	E-Z		
	X-Y	**	**
	X-F	**	**
	X-L	**	**

APPENDIX F (CONTINUED):

Data Set #2

Character	Group Comparison	Significance: ** = significant at alpha=0.01	
		a	b
	X-S	**	**
	X-Z	**	**
	Y-F		
	Y-L		
	Y-S	**	**
	Y-Z		
	F-L		
	F-S	**	**
	F-Z		
	L-S	**	**
	L-Z		
	S-Z	**	
12	E-X	**	**
	E-Y		
	E-F		
	E-L		
	E-S		
	E-Z		
	X-Y		
	X-F		
	X-L		
	X-S		
	X-Z		
	Y-F		
	Y-L		
	Y-S		
	Y-Z		
	F-L		
	F-S		
	F-Z		
	L-S		
	L-Z		
	S-Z		
13	E-X	**	**
	E-Y	**	**
	E-F	**	**
	E-L	**	**
	E-S	**	**
	E-Z		
	X-Y		

APPENDIX F (CONTINUED):

Data Set #2

Character	Group Comparison	Significance: ** = significant at alpha=0.01 a	b
	X-F		
	X-L		
	X-S		
	X-Z		
	Y-F		
	Y-L		
	Y-S		
	Y-Z		
	F-L		
	F-S		
	F-Z		
	L-S		
	L-Z		
	S-Z		
14	E-X	**	**
	E-Y		
	E-F		
	E-L		
	E-S		
	E-Z		
	X-Y	**	
	X-F	**	**
	X-L	**	**
	X-S	**	**
	X-Z	**	**
	Y-F		
	Y-L		
	Y-S		
	Y-Z		
	F-L		
	F-S		
	F-Z		
	L-S		
	L-Z		
	S-Z		
15	E-X	**	**
	E-Y	**	**
	E-F		
	E-L		
	E-S		
	E-Z		
	X-Y		

APPENDIX F (CONTINUED):

Data Set #2

Character	Group Comparison	Significance: ** = significant at alpha=0.01	
		a	b
	X-F		
	X-L		**
	X-S	**	**
	X-Z	**	**
	Y-F		
	Y-L		
	Y-S		
	Y-Z		
	F-L		
	F-S		
	F-Z		
	L-S		
	L-Z		
	S-Z		
16	E-X		
	E-Y	**	**
	E-F	**	
	E-L		
	E-S		
	E-Z		
	X-Y	**	**
	X-F		
	X-L	**	
	X-S		
	X-Z	**	
	Y-F	**	**
	Y-L	**	**
	Y-S	**	**
	Y-Z		
	F-L	**	**
	F-S		
	F-Z	**	**
	L-S		
	L-Z		
	S-Z	**	**

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