Evolutionary Biology of *Trillium* and Related Genera (Trilliaceae) II. Cladistic Analyses on Gross Morphological Characters, and Phylogeny and Evolution of the Genus *Trillium*

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Abstract Cladistic analyses were conducted based upon 22 gross morphological and one floral odor characters for 42 *Trillium* species, one *Kinugasa*, one *Daiswa*, and two *Paris* species of the Trilliaceae, using *Daiswa* and *Paris* as outgroups. The cladistics provided an informative result with regard to the affinity and phylogeny of the taxa referred to the genus *Trillium* and related genera in the Trilliaceae, suggesting that *Trillium* is composed of four major infrageneric groups: (1) the pedicellate-flowered group represented by the Erectum group including 13 species, (2) the Undulatum group, a loosely clustered paraphyletic group including five species, (3) the Govanianum group consisting of only two species, and (4) the Sessile group including 22 species. The monophyly of the Sessile group is very evident. The results are concordant with the earlier results of the RFLP analysis of *cp*DNA conducted by us. It is noteworthy, however, that the pedicellate-flowered group of *Trillium* is not simply monophyletic, as was assumed previously, but instead consists of at least three to four distinct groups as noted above. The implications and limitations of using distantly related genera, such as *Daiswa* or *Paris* as outgroups, in the cladistic analysis were also argued.

Key words: Arcto-Tertiary element, cladistic analysis, *Daiswa*, *Kinugasa*, *Paris*, phylogeny, *Trillium*, Trilliaceae.

The genus *Trillium* (Trilliaceae *sensu* Dahlgren et al., 1985; Hutchinson, 1959; or Liliaceae *sensu* Krause, 1930) is known to include ca. 42 species. The genus is referred to as one of the so-called Arcto-Tertiary elements, showing a typical disjunct distribution in wide areas of eastern Asia. *Trillium* spp. range from the northern Pacific regions to the Himalayan mountains throughout the Islands of Japan and in temperate or boreal woodlands of western and eastern North America (Gray, 1846, 1860; Li, 1952; Axelrod, 1966; Graham, 1972; Tiffney, 1985; Samejima and Samejima, 1987; Ohara, 1989).

In connection with a series of evolutionary-biological studies of the genus *Trillium* (Kawano et al., 1986, 1992; Ohara, 1989; Ohara and Kawano, 1986a, b; Ohara and Utech, 1986, 1988; Ohara et al., 1990), we have analyzed the phylogeny of *Trillium*, using 24 species, by means of restriction fragment length polymorphism (RFLP) analyses of chloroplast DNA (*cp*DNA) (Kato et al., 1995a), and also have attempted to analyze the phylogenetic status of this genus together with several other genera in the Trilliaceae based upon the

sequence data of *rbc*L gene of *cp*DNA (Kato et al., 1995b). These earlier studies shed light upon the phylogenetic relationships among species of *Trillium* and other closely related genera in the Trilliaceae.

In the present study we have conducted cladistic analyses based upon the gross morphological and floral fragrance characters to determine the systematic status and phylogeny of the presently known 42 *Trillium* species (Samejima and Samejima, 1987) and furthermore have attempted to compare the results of cladistic analyses with those of molecular systematic analyses (Kato et al., 1995a) and comparative life history studies (Ohara, 1989; Kawano et al., 1992). All these data and evidence available at present enable us to synthesize the phylogeny and evolution of this most interesting genus of the Arcto-Tertiary origin.

Materials and Methods

1. *Species and Characters Studied*
Fourty two *Trillium* species were examined in the present study (Table 1). In the present cladistic analyses, one species each of *Kinugasa* and *Daiswa* and two *Paris* species were also included. The 22 gross morphological and one floral odor characters were chosen, and their character states were determined for each charac-

Table 1. Species studied.

Species	Distribution
Trillium apetalon	Japan
T. kamtschaticum	Japan, Korea, China, Kamchatka
T. tschonoskii	Japan, Korea,China, Himalayas, Taiwan
T. smallii	Japan
T. taiwanense	Taiwan
T. govanianum	Himalayas
T. cernuum	eastern North America
T. erectum	eastern North America
T. flexipes	eastern North America
T. rugelii	eastern North America
T. vaseyi	eastern North America
T. sulcatum	eastern North America
T. persistens	eastern North America
T. undulatum	eastern North America
T. catesbaei	eastern North America
T. grandiflorum	eastern North America
T. ovatum	western North America
T. nivale	eastern North America
T. rivale	western North America
T. pusillum var. *ozarkanum*	eastern North America
T. pusillum var. *pusillum*	eastern North America
T. recurvatum	eastern North America
T. lancifolium	eastern North America
T. sessile	eastern North America
T. decumbens	eastern North America
T. underwoodii	eastern North America
T. decipiens	eastern North America
T. reliquum	eastern North America
T. discolor	eastern North America
T. stamineum	eastern North America
T. maculatum	eastern North America
T. foetidissimum	eastern North America
T. cuneatum	eastern North America
T. luteum	eastern North America
T. ludovicianum	eastern North America
T. gracile	eastern North America
T. viride	eastern North America
T. viridescens	eastern North America
T. petiolatum	western North America
T. albidum	western North America
T. chloropetalum	western North America
T. angustipetarum	western North America
T. kurabayashii	western North America
Kinugasa japonica	Japan

Table 1. continued

Species	Distribution
Paris verticillata	Japan, Korea, China, Siberia
P. tetraphylla	Japan
Daiswa polyphylla	South eastern Asia

ter as follows, based upon our own observations and in reference to several previous monographic as well as floristic works (Fernald, 1950; Munz, 1965; Ohwi, 1965; Freeman, 1975; Samejima and Samijima, 1987; Kawano, 1994) (Table 2).

(i) *Root systems*:

The rhizomes of *Trillium* are normally yellowish brown or dark brown, stout with scaly leaf scars; only in a few exceptional sessile-flowered species, we can see long-creeping slender rhizomes, which obviously represent a derived character state. Interestingly, offshoot formation can often be seen in such rhizomes (Ohara and Utech, 1986; Kawano et al., 1992; Kawano, 1994) as well as in stout forms.

1) Rhizome: stout (0); slender (1); long-creeping, rarely with forks (2).

(ii) *Leaf characters*:

The leaf characters of *Trillium* are very diverse. The eastern Asiatic diploid pedicellate species, *T. kamtschaticum*, and eastern North American pedicellate species such as *T. erectum, T. sulcatum*, etc., which are assumed to retain some ancestral character states (Samejima and Samejima, 1987), possess large rhombic, broad-rhombic or broad-ovate leaves, with sharply-pointed leaf tip. The broadest part of the lamina is at 1/2 or 1/3 from the leaf base. Often leaves are sharply mottled, notably in some sessile-flowered species of North America. Most of the *Trillium* species' leaves are sessile, but short- or long-petiolate leaves are occasionally found in some species. Thus, a petiolate character no doubt represents an apomorphic character state.

2) Petiole: absent or very short (<2mm)(0); present (2–20mm)(1); long (>20mm) (2).

3) The broadest portion of leaves: 1/2 (0); 1/3 (1); 2/3 (2); 4/5 (3)

4) Leaf tip: acute or acuminate (0); obtuse (1); round (2).

5) Leaf shape: rhombic (0); broad-ovate (1); round-ovate (2); ovate (3); round (4); oblong-ovate (5); lanceolate (6); oblanceolate (7).

6) Leaf color: non-mottled (0); mottled (1).

(iii) *Floral and fruit characters*:

Floral characters have been used as the major basis for the definition of taxa in *Trillium*. The pedicel character—its presence or absence—first attracted taxonomists' attention. The subgenus *Trillium*, consisting of all pedicellate-flowered species, and subgenus

Table 2. The characters used in the cladistic analysis. The plesiomorphic states are in general indicated by 0 and apomorphic states by 1, 2, 3, etc.

Characters and states	Character types
1. Rhizome: stout (0); slender (1), long creeping (2)	ordered
2. Petiole length: <2mm (0); 2–20mm (1); >20mm (2)	ordered
3. Broadest part of leaves: 1/2 (0); 1/3 (1); 2/3 (2); 4/5 (3)	0 (1, 2 (3))*
4. Leaf tip: acute (0); obtuse (1); round (2)	ordered
5. Leaf shape: rhombic (0); broad-ovate (1); round-ovate (2); ovate (3); round (4); oblong-ovate (5); lanceolate (6); oblanceolate (7)	0 (1 (2, 3 (4, 5 (6, 7))))*
6. Leaf color: non-mottled (0); mottled (1)	ordered
7. Pedicel (at anthesis): erect (0); declined (1); lacking (2)	0 (1, 2)*
8. Pedicel (fruiting): erect (0); vertical (1); declined (2); lacking (3)	0 (1 (2), 3)*
9. Sepal: erect (0); vertical (1); reflexed or recurved (2)	ordered
10. Sepal shape: ovate (0); oblong-ovate (1); narrow ovate (2); linear (3)	ordered
11. Petal color: white (0); white to pink (1); pink tinted (2); pink spotted (3); reddich purple (4); yellow (5); greenish yellow (6); greenish purple or dark purple (7); petal lacking (8)	unordered
12. Petal: patent (0); reflexed or recurved (1); erect (2); erect, slightly twisted (3); twisted (4); petal lacking (5)	0 (1, 2, 3(4), 5)*
13. Petal shape: ovate (0); lanceolate (1); oblanceolate (2); linear-lanceolate (3); broad-ovate (4); round (5); linear (6); petal lacking (7)	0 (1 (2 (3,)), 4(5), 6, 7)*
14. Anther dehiscence: lateral (0); extrorse (1); introrse (2)	0 (1, 2)*
15. Filament/anther: >0.8 (0); 0.4–0.7 (1); <0.3 (2)	ordered
16. Stamen connective 1: erect (0); recurved (1)	ordered
17. Stamen connective 2: not-protrusive (0); protrusive (1)	ordered
18. Stigma: shortly-beaked (0); tristylous (1); enlarged-stigma (2)	ordered
19. Ovary: globose (0); angular-ovoid (1); ovoid (2); conical (3)	0 (1, 2, 3)*
20. Flower order: non-fragrant (0); fragrant (1); strong fetid (2)	0 (1, 2)*
21. Fruit: berry (0); capsule (end+myr) (1); capsule (myr) (2)	0 (1, 2)*
22. Leaf number: 3 (0); >4 (1); 4 (2)	0 (1, 2)*
23. Sepal number: 3 (0); >4 (1); 4 (2)	0 (1, 2)**

* User-defined character type (cf. PAUP 3.1 user's manual)

Phyllantherum, consisting of all sessile-flowered species can be diagnosed primarily by this character (Freeman, 1975). The pedicel characters are also variable at the flowering stage and the fruiting stage. Some species have erect pedicels at both flowering and fruiting time, but in others pedicels may become vertical or even strongly declinate at fruiting time. Several eastern North American species possess typical declinate pedicels at both flowering and fruiting time. All the other floral characters are related to the pedicel states to a certain extent.

Character states in pedicel, petal, androecium and gynoecium are obviously related to the levels of differentiation in the pollination and breeding systems of each species. The fruit and seed characters are strongly related to the dispersal mechanisms of propagules (Berg, 1958; Kawano et al., unpubl. and in preparation).

7) Pedicel at the flowering stage: erect (0); declined (1); lacking (2).

8) Pedicel at the fruiting stage: erect (0); vertical (1); declined (2); lacking (3).

9) Sepal angles: erect (0); vertical (1); reflexed or recurved (2).

10) Sepal shape: ovate (0); oblong-ovate (1); narrow-ovate (2); linear (3).

11) Petal color: white (0); white to pink (1); pink-tinted (2); pink-spotted (3); reddish purple (4); yellow (5); greenish yellow (6); greenish or dark purple (7); petal lacking (8) (scores given, but unordered).

12) Petal angles: patent (0); reflexed or recurved (1); erect (2); erect, slightly twisted (3); twisted (4); petal lacking (5).

13) Petal shape: ovate (0); lanceolate (1); oblanceolate (2); linear-lanceolate (3); broad-ovate (4); round (5); linear (6); petal lacking (7).

14) Anther dehiscence: lateral (0); extrorse (1); introrse (2).

15) Filament/anther ratio: >0.8 (0); 0.4–0.7 (1); <0.3 (2).

16) Stamen connectives: erect (0); recurved (1).

17) Stamen connectives: not-protrusive (0); protrusive

(1).

(18) Stigma: shortly-beaked (0); tristylous (1); enlarged (2).

(19) Ovary: globose (0); angular-ovoid (1); ovoid (2); conical (3).

(20) Floral odor: non-fragrant (0); fragrant (1); strong fetid (2).

(21) Fruit: berry (0); capsule (endozoochory plus myrmecochory) (1); capsule (myrmecochory)(2).

(iv) *Basic structures of floral and vegetative organs*:
All reproductive and vegetative organs of mature *Trillium* plants, i.e., floral parts (sepals, petals, numbers of stamens and styles) and leaves are trimerous, except for some abnormalities. However, as was described in detail in our series of comparative life history studies, seedlings and juveniles of *Trillium* are all single-leaved; then these juveniles switch from this single-leaf state to trimerous states (Samejima and Samejima, 1987; Ohara, 1989; Kawano et al., 1992; Kawano, 1994). However, interestingly enough, genera that are assumed to be related to *Trillium*, such as *Paris*, *Daiswa* and *Kinugasa* (Kato et al., 1995a, b) are entirely distinct in their basic organ architecture. Indeed, all *Paris* species possess tetramerous or hexamerous floral parts. *Daiswa* and *Kinugasa* show even more pronouncedly specialized character states and possess more numerous floral and vegetative parts, ranging from 8 to 10. Thus, we judge here that all these character states found in genera related to *Trillium* are derived, under extremely specialized conditions, from some already extinct ancestral groups of the Trilliaceae. Such circumstances make it very difficult to select an outgroup to root the tree in the cladistic analysis. However, taking of this shortcoming into account, we have used *Paris* and *Daiswa* as outgroups in the present analysis.

(22) Leaf number: 3 (0); >4 (1); 4 (2).

(23) Sepal number: 3 (0); >4 (1); 4 (2).

2. Cladistic Analyses

Twenty-three characters with a total of 89 states (Table 2) were scored (Table 3). Cladistic parsimony analyses were carried out using Swofford's package PAUP Ver. 3.1.1 (Swofford, 1993), according to the methods of Farris (1989). The random addition sequences option in the heuristic search was used to obtain islands of equally optimal (parsimonious) trees. The islands were searched by 100 random replications, using the option MULPAR (storage of equally parsimonious tree), and TBR (tree bisection-reconnection) branch swapping. In addition, we defined the polalities of character states using the option of user-defined character type (Table 2) because *Paris* species have several apomorphic characters. Character-state changes were placed on trees using the accelerated transformation method (ACCTRAN).

Paris verticillata and *Daiswa polyphylla* were used as outgroups. However, one of the difficulties using *Paris*

Table 3. Data matrix for the phylogenetic analysis of *Trillium*. Unknown states are coded as ?

Species	Character no. 11111111112222 12345678901234567890123
Trillium apetalon	00000000008570100010000
T. kamtschaticum	00000000010000100030000
T. tschonoskii	0000000001000010000000 4
T. smallii	00000000004050100000000
T. taiwanense	0100300020406000001??00
T. govanianum	01103000234160100120000
T. cernuum	00000012010000000100000
T. erectum	0000000101100010001100 4
T. flexipes	00000012020000100000001
T. rugelii	00000012212100100001000
T. vaseyi	00000012014040100111000
T. sulcatum	00000001014000100011000
T. persistens	00005000012000100110100
T. undulatum	01103000022000000120100
T. catesbaei	00003012221100020101000
T. grandiflorum	00001001011000100000200
T. ovatum	0010100111100010010020 4
T. nivale	00115000120000000100100
T. rivale	02103002003040000020100
T. pusillum var. *ozarkanum*	10016000021010000020100
T. pusillum var. *pusillum*	10016023021010000020100
T. recurvatum	1100302321622211121020 1 7
T. lancifolium	1000602321621201121020 1 7
T. sessile	0000202312621220121220 1 7
T. decumbens	00121123117311201202200
T. underwoodii	0010312322631020100220 7
T. decipiens	0000312311632020103120 7
T. reliquum	00012123117312201202200
T. discolor	0000102311532220120120 1
T. stamineum	0010212311641120123220 7
T. maculatum	00003123127322200231200
T. foetidissimum	0000112312631220123220 7
T. cuneatum	0000312312632020123220 7
T. luteum	00001123115322200231200
T. ludovicianum	0001512312623020123220 7
T. gracile	0001312312623220123220 7

Table 3. continued

Character no.	11111111112222
Species	12345678901234567890123
T. viride	00005023127232201201200 1
T. viridescens	00003023127230201221200 1
T. petiolatum	02024023217210101230200
T. albidum	00001023120220200231200 1
T. chloropetalum	00001123127222201201200
T. angustipetalum	00001123127212201202200
T. kurabayashii	00005123127212201202200
Kinugasa japonica	00307000110260000120011
Paris verticillata	20205000115160001120011 1
Paris tetraphylla	20005000118570000120022
Daiswa polyphylla	01205000125060101120111 2

and *Daiswa* as outgroups is that the character states found in these species are all extremely specialized and discontinuous from those of any present-day *Trillium* species, obviously representing the derived states from several ancestral, but now extinct species in the proto-Trilliaceae.

Results and Discussion

1. *Results of cladistic analyses*
Cladistic analyses of the data in Table 3 gave 5900 trees, each 193 steps long, with a consistency index (CI) of 0.342, a homoplasy index (HI) of 0.658, a retention index (RI) of 0.723, and a rescaled consistency index of (RC) of 0.247 (Farris, 1989). One of the equally parsimonious cladograms is shown in Fig. 1. A 50% majority rule consensus tree of 5900 equally parsimonious trees is given in Fig. 2.

The tree topologies shown in Figs. 1 and 2 gave some informative pictures of the infrageneric groups of *Trillium*. It is now very evident that the genus consists of four major groups, the three pedicellate-flowered groups and one sessile-flowered group. According to the present cladistic analyses, two Asiatic pedicellate species, *T. govanianum* and *T. taiwanense*, are paraphyletic to the Sessile group of *Trillium*, but we are not certain whether or not this tree topology reflects the true affinities and phylogenetic status of these two extremely specialized species in their character states. The pedicellate group is further subdivided into subgroups, a group including eight erect-flowered species and five declinate-flowered species (hereinafter called

the Erectum group), and a loosely clustered paraphyletic group including five pedicellate species, i.e., *T. persistens, T. undulatum, T. rivale, T. pusillum*, and *T. nivale* (hereafter called the Undulatum group). This latter subgroup has not been recognized in any previous taxonomic studies, and the present result, together with those of molecular systematic analyses on *cp*DNA (Kato et al.,1995a), suggests that the origin of the Undulatum group may be somewhat different or may even show that the pedicellate group is not simply monophyletic in origin.

As described in the foregoing section of the character state scoring (Tables 2 and 3), changes in character states of *Trillium* are not very simple. The pedicel has been used as a character of diagnostic value in the infrageneric grouping of *Trillium*. However, there is no doubt that the sessile-flowered group is monophyletic, and perhaps its origin was once from some ancestral group in the evolutionary history of the genus, although a considerable degree of specialization has occurred since the differentiation from the pedicellate-flowered ancestors. The cladograms shown in Figs. 1 and 2 clearly demonstrate the status of the sessile-flowered group, specifically differentiated in North America. Perhaps due to many homoplasies involved, the resolution of cladistic analyses for species of the Sessile-group is not very high, and yet there is a good deal of informative evidence in the tree constructed here (cf. Fig. 1). *T. recurvatum* and *T. lancifolium* are specialized in their root system characters. *T. viride*, a species ranging in eastern hilly parts of Missouri and western Illinois, and *T. viridescens* of the Ozark mountains certainly form a pair of sister species. *T. petiolatum* of the West Coast is highly specialized in many floral and vegetative characters, thus being paraphyletic to the major members of the Sessile group.

To sum up: the present results clearly indicate that the genus *Trillium* consists of four pedicellate infrageneric and one sessile groups, i.e., the Govanianum, Erectum, Grandiflorum, Undulatum, and the Sessile group.

2. *Evaluation of Changes in Character States*
The present cladistic analyses provided some interesting results concerning the evolutionary changes of character states in *Trillium*. As has been repeatedly stated, the pedicel is an important character used in the taxonomic delimitation of infrageneric taxa in *Trillium*. Figure 3 demonstrates the frequencies of changes in the character state that occurred in this particular trait. A sessile-flowered form of *T. pusillum* (called var. *virginianum* [Fernald, 1943; Reed, 1982]; pedicellate-flowered plants called var. *pusillum* and var. *ozarkanum*) is no doubt a homoplasy which has differentiated rather recently in several eastern populations of this species and seems not to have any direct phylogenetic relationships to the other major sessile-flowered group in-
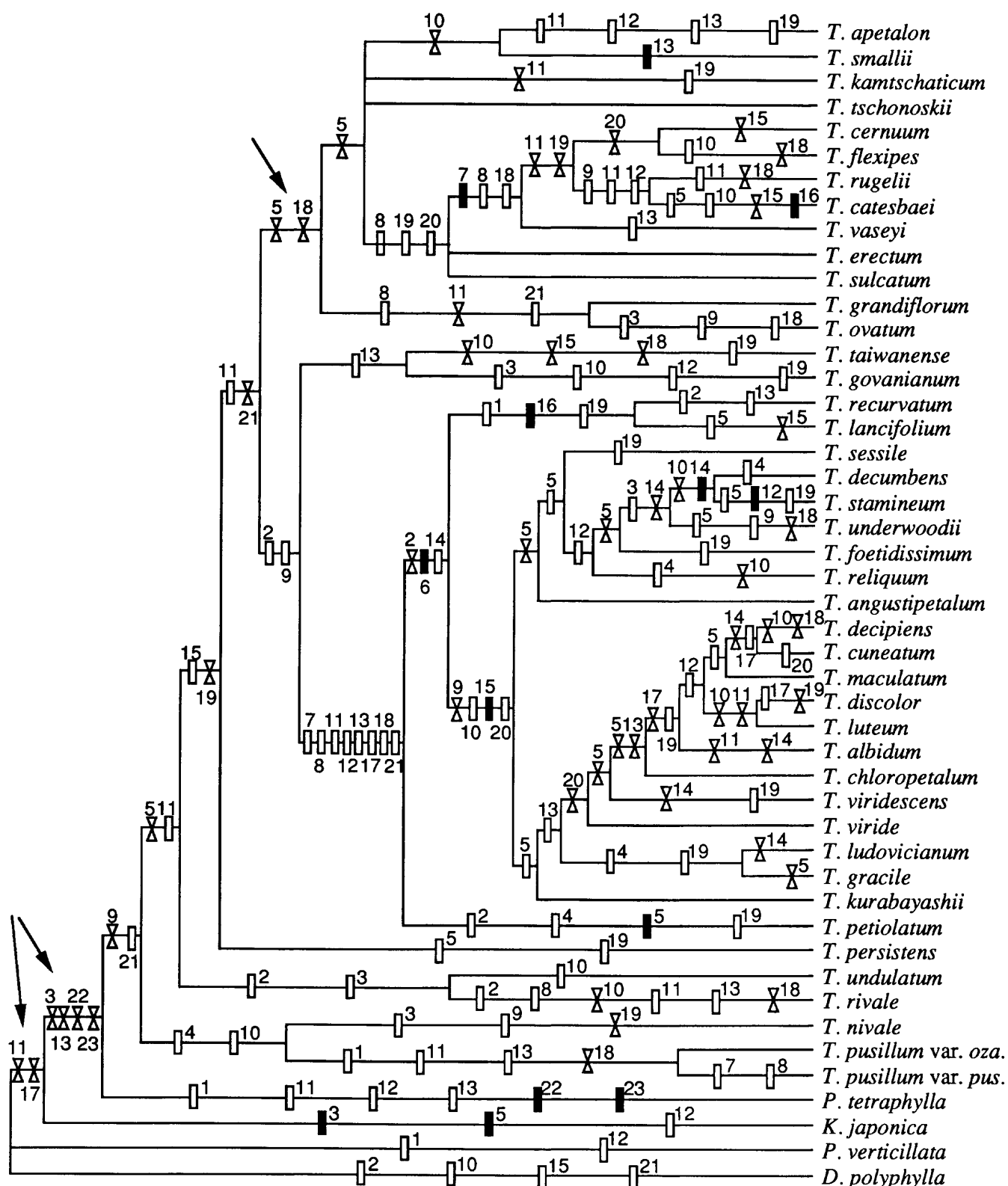
Fig. 1. One of the 5900 equally parsimonious cladograms of the *Trillium* constructed from the data matrix (Table 3). Changes of character states were estimated by ACCTRAN optimization using PAUP ver. 3.1.1. *Paris verticillata* and *D. polyphylla* were used as outgroups. Numbers indicate the character states. Open bars represent homoplastic character changes. Black bars represent the apomorphic characters. ⋈ =reversal. Tree legth=193; consistency index (CI)=0.34; homoplasy index (HI)=0.66; retention index (RI)=0.72. Arrows showing some critical reversal changes (see text).

52 ┌─ T. apetalon
   └─ T. smallii
T. kamtschaticum
T. tschonoskii
52 ┌─ T. cernuum
   └─ T. flexipes
100 ┌─ T. rugelii
84 └─ T. catesbaei
T. vaseyi
84 ┌─ T. erectum
   └─ T. sulcatum
100 ┌─ T. grandiflorum
    └─ T. ovatum
100 ┌─ T. taiwanense
    └─ T. govanianum
100 ┌─ T. recurvatum
    └─ T. lancifolium
T. sessile
100 ┌─ T. decumbens
    └─ T. stamineum
61 ┌─ T. underwoodii
   └─ T. decipiens
54 T. cuneatum
T. reliquum
100 ┌─ T. discolor
66 └─ T. luteum
64 T. maculatum
64 T. albidum
52 T. chloropetalum
64 ┌─ T. viride
   └─ T. viridescens
T. foetidissimum
100 ┌─ T. ludovicianum
    └─ T. gracile
T. angustipetalum
T. kurabayashii
T. petiolatum
T. persistens
100 ┌─ T. undulatum
    └─ T. rivale
100 T. nivale
100 ┌─ T. pusillum var. oza.
    └─ T. pusillum var. pus.
P. tetraphylla
K. japonica
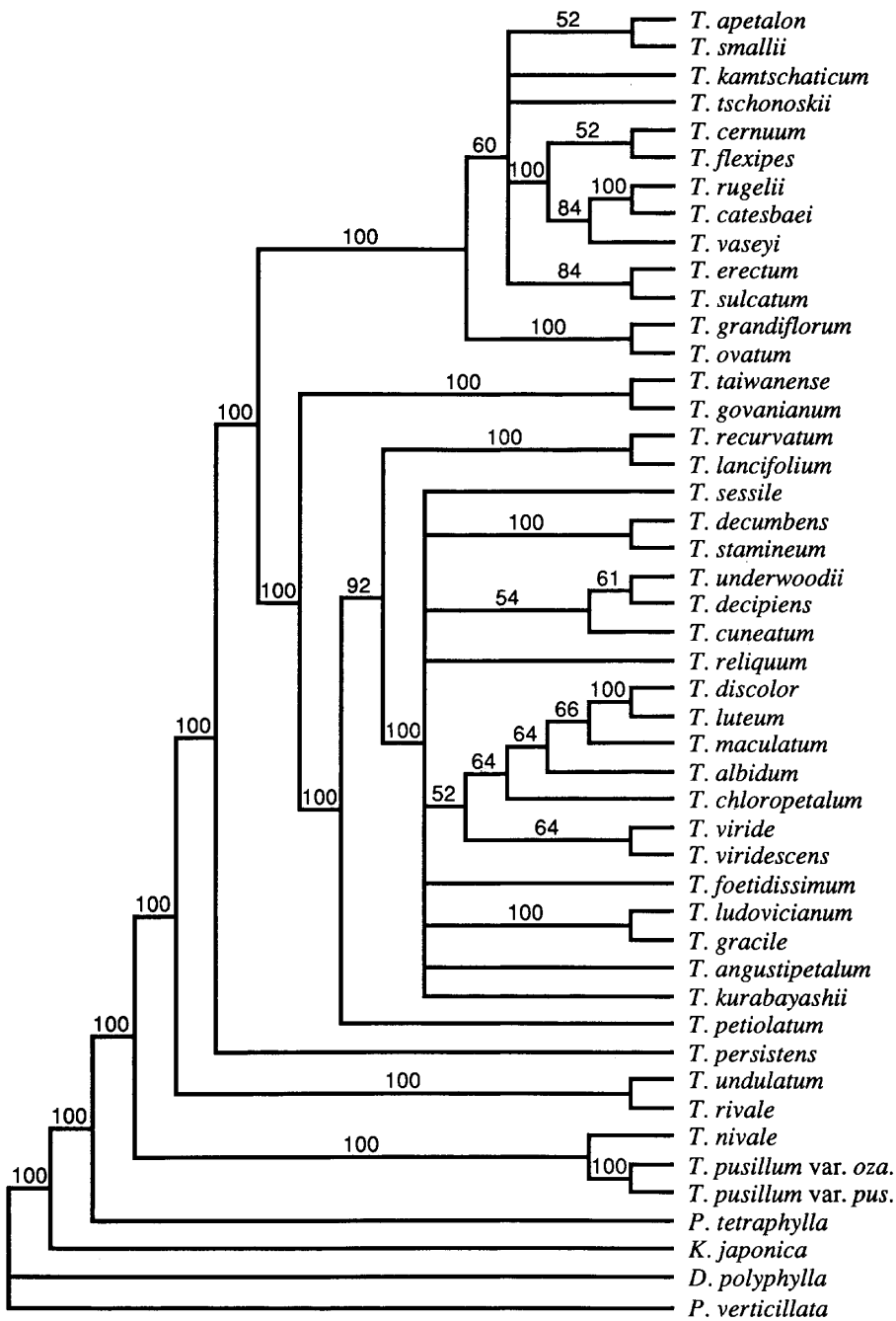D. polyphylla
P. verticillata

Fig. 2. 50% majority rule consensus tree of 5900 equally parsimonious trees. The numbers indicate the group frequencies.

cluding ca. 20 species. Indeed, *T. pusillum* populations in North Carolina are known to often comprise both sessile- and pedicellate-flowered plants, but pedicellate forms are very rare in Virginia and Maryland; while all plants in South Carolina are pedicellate. There is evidence that a cline from South to North along the Coastal Plain in which pedicels gradually disappear, and the southern populations are probably older in origin (Samejima and Samejima, 1987; Cabe, 1995; Cabe and Werth, 1995; Freeman, pers. comm.). The degrees of declination of the pedicels among the eastern North American pedicellate species are quite variable in different species and also at the different stages of fruit maturation of the same species, notably *T. erectum* and *T. rivale*.

Reproductive characters, such as petal shape, petal color, manner of flowering, and the development of signal characters attracting pollinators, show rather complex correlated patterns of differentiation. We assumed that the plesiomorphic character state in the flower color is white and non-fragrant in *Trillium*. It is thus possible that in these white flowered species only visual signals play major roles in attracting pollinators. It is also very obvious that the differentiations of flower color are homoplastic in many possible directions, from white to pinl, red, purplish red, chocolate, greenish brown, green and yellow, or vice versa. Thus, in the present analysis, the flower color character was computed unordered. At present, only limited data are available as to floral color chemistry of *Trillium*, e.g.,
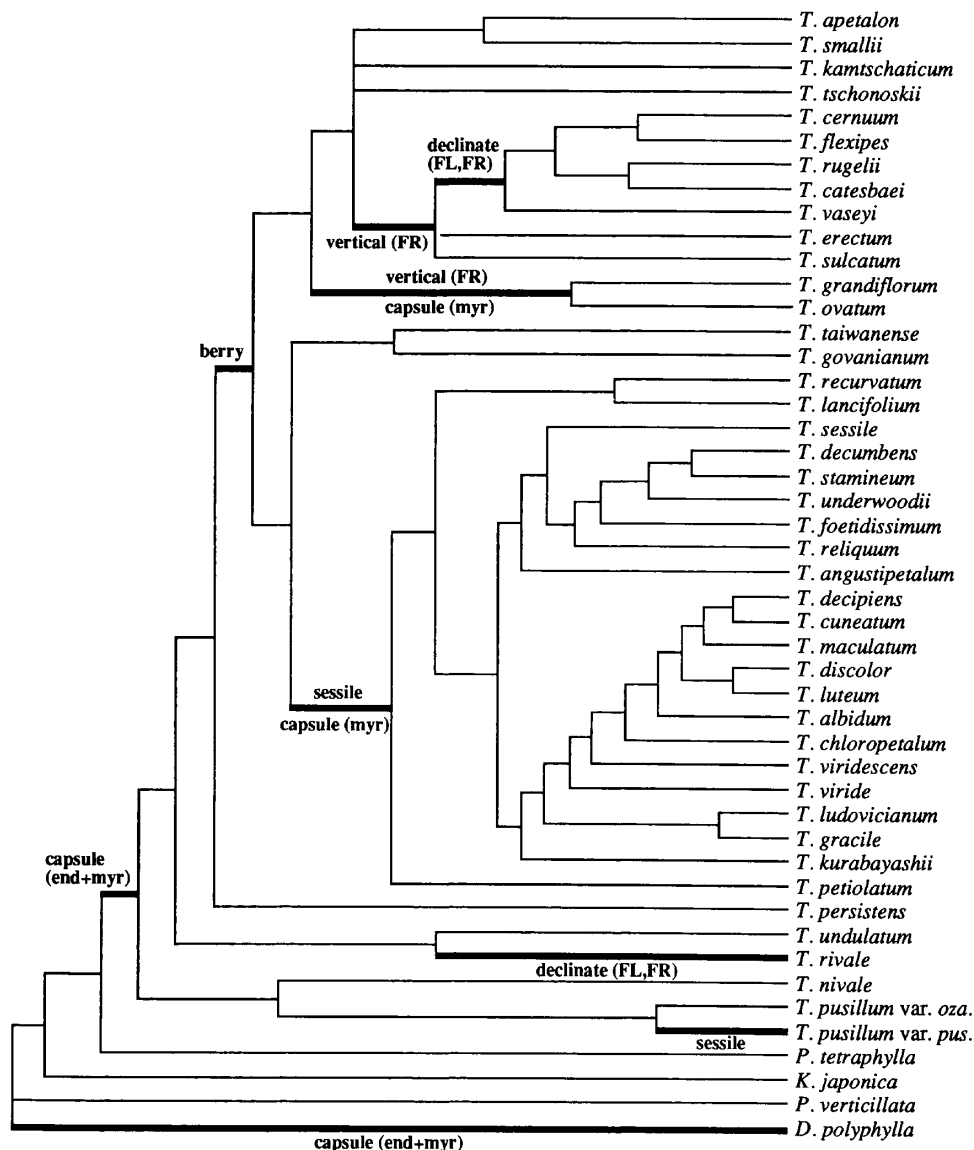
Fig. 3. Evolutionary changes found in pedicel and fruit characters. Plesiomorphic character states are assumed to be erect-peduncle and berry. Changes of character states were estimated by ACCTRAN optimization using PAUP ver. 3.1.1. FL, flowering time; FR, fruiting time; end, endozoochory; myr, myrmecochory.

both pedicellate and sessile species possess two similar anthocyanin components (Kozuka, 1963 and unpubl.; Murrell, 1969). Simultaneously, various biosynthetic pathways of volatile substances have perhaps differentiated in relation to pollination and breeding systems.

It is noteworthy that most of the sessile-flowered *Trillium* species possess considerably large anthers with various dehiscence types, such as introrse, lateral or extrorse. It is also notable that the stigma surfaces of most of these species are enlarged, facilitating the establishment of pollen grains on their surface. Such character states may be strongly related to the breeding systems of these species: for example, a flower with extrorse anther dehiscence and strongly recurved stigma surface, producing a strong fetid odor from laterally open but strongly twisted narrow oblanceolate petals, as found in *T. stamineum*, no doubt represents a xenogamous breeding system, and indeed this has been confirmed by bagging and hand-pollination ex-

periments in the field population of *T. stamineum* (Ohara, Takasu and Kawano, unpubl.). On the other hand, lateral or introrse dehiscent anthers and tightly closed stigma surface with non-fragrant flowers tend to promote inbreeding. All these floral characters predominantly found in the sessile-flowered *Trillium* species seem to have differentiated in relation to changes in pollinators from beetles, small bees, etc. to dung flies and some other rather short-ranging insects. These insects may not be very efficient pollinators in terms of pollen transfer among flowering individuals, but they still may secure the possibility of outbreeding within a population to a considerable extent.

Large sterile and fertile individuals of all *Trillium* species always bear three leaves at the top of aerial stems. The shapes and sizes of leaves are, however, quite variable in different species. The leaf is where photosynthesis occurs, but simultaneously is where transpiration occurs, lowering leaf temperature exposed to direct solar radiation. In this respect, leaf size (or leaf

area) is an important character controlling the balance between photosynthetic efficiency and leaf temperature. Naturally, plants that grow in deep shade bear larger leaves; in contrast, those that grow in dry rocky or gravelly habitats bear smaller and/or narrower leaves. Thus, we can recognize characteristic adaptive radiations and homoplasy in this particular character, reflecting selection regimes in their habitats (Fig. 4). As

was first assumed, it seems reasonable to consider that rhombic, broad-rhombic, or broad-ovate leaves, as found in some diploid species of the pedicellate-flowered group (*T. erectum, T. sulcatum*, and *T. kamtschaticum*, etc.), represent a plesiomorphic character state in *Trillium*. (Table 2).

Homoplasy indices (HI)(Table 4) clearly demonstrate the degrees of homoplasy detected in 22 gross morpho-
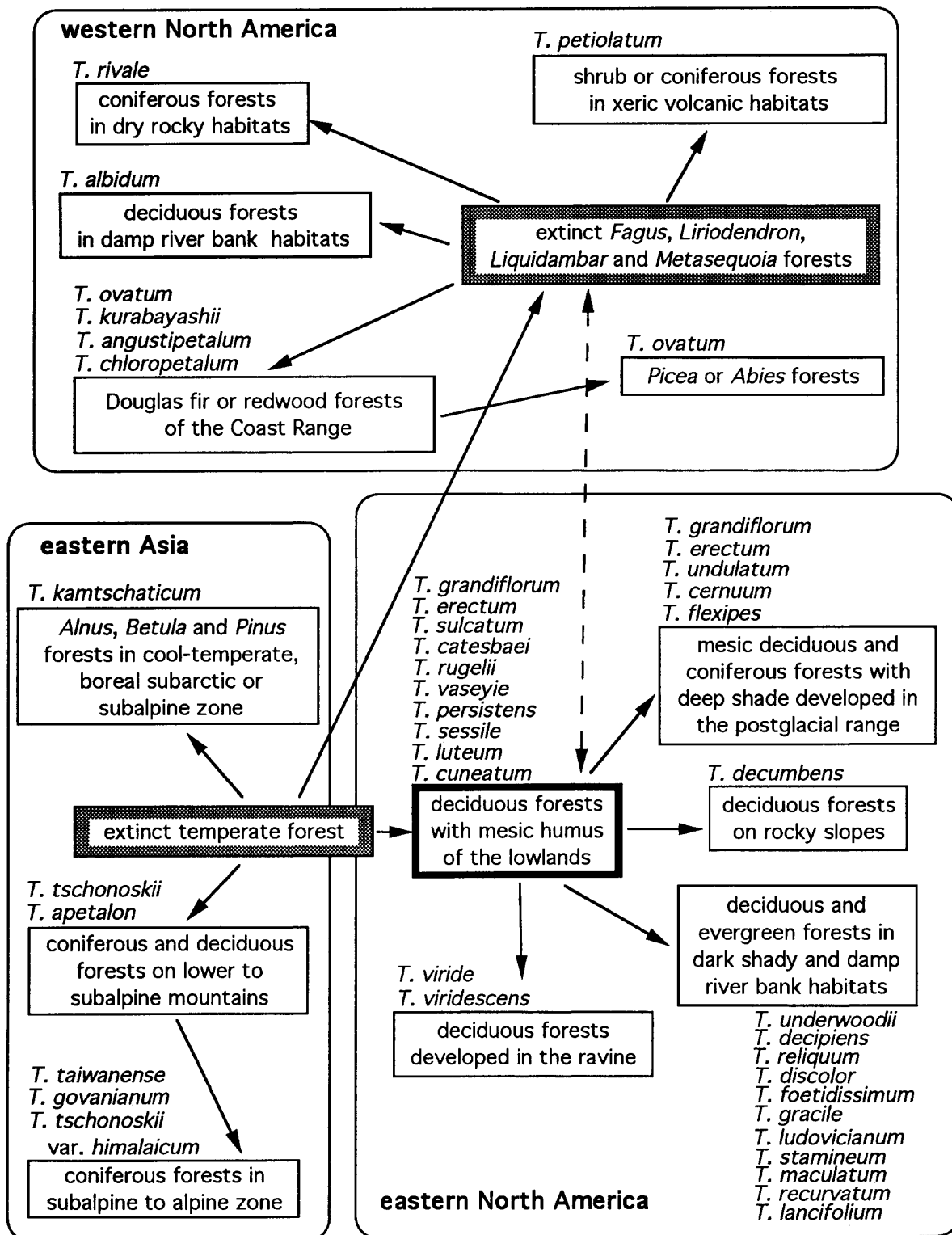
western North America

T. rivale

coniferous forests
in dry rocky habitats

T. petiolatum

shrub or coniferous forests
in xeric volcanic habitats

T. albidum

deciduous forests
in damp river bank habitats

extinct *Fagus, Liriodendron,
Liquidambar* and *Metasequoia* forests

*T. ovatum
T. kurabayashii
T. angustipetalum
T. chloropetalum*

Douglas fir or redwood forests
of the Coast Range

T. ovatum

Picea or *Abies* forests

eastern Asia

T. kamtschaticum

Alnus, Betula and *Pinus*
forests in cool-temperate,
boreal subarctic or
subalpine zone

*T. grandiflorum
T. erectum
T. sulcatum
T. catesbaei
T. rugelii
T. vaseyie
T. persistens
T. sessile
T. luteum
T. cuneatum*

*T. grandiflorum
T. erectum
T. undulatum
T. cernuum
T. flexipes*

mesic deciduous and
coniferous forests with
deep shade developed in
the postglacial range

extinct temperate forest

deciduous forests
with mesic humus
of the lowlands

T. decumbens

deciduous forests
on rocky slopes

*T. tschonoskii
T. apetalon*

coniferous and deciduous
forests on lower to
subalpine mountains

*T. viride
T. viridescens*

deciduous forests
developed in the ravine

deciduous and
evergreen forests in
dark shady and damp
river bank habitats

*T. taiwanense
T. govanianum
T. tschonoskii*
var. *himalaicum*

coniferous forests in
subalpine to alpine zone

eastern North America

*T. underwoodii
T. decipiens
T. reliquum
T. discolor
T. foetidissimum
T. gracile
T. ludovicianum
T. stamineum
T. maculatum
T. recurvatum
T. lancifolium*

Fig. 4. Ecological radiations in the habitats of *Trillium*.

Table 4. Homoplasy indices (HI) for each character.

Character no.*	1	2	3	4	5	6	7	8	9	10	11	12
HI	0.67	0.71	0.57	0.71	0.67	0	0.33	0.57	0.78	0.80	0.53	0.58

Character no.*	13	14	15	16	17	18	19	20	21	22	23
HI	0.50	0.71	0.71	0.33	0.80	0.85	0.83	0.71	0.60	0	0*

* See Table 2

logical and one floral odor characters examined in the present study.

General Discussion

1. *Evaluation of* Trillium *Phylogeny*

The genus *Trillium* no doubt represents one of the best studied genera in the Trilliaceae or Liliaceae s. *lat.* (Krause, 1930) from various standpoints, including gross morphology (Berg, 1958; Freeman, 1975; Samejima and Samejima, 1962, 1987), palynology (Takahashi, 1982, 1983, 1984), cytogenetics and karyology (Haga and Kurabayashi, 1954; Dyer, 1964a, b, c; Utech, 1980), population genetics (Kurabayashi, 1958; Fukuda and Channell, 1975; Fukuda and Grant, 1980), artificial hybridization (Haga and Channell, 1982), comparative life histories (Kawano et al., 1986, 1992; Ohara, 1989; Ohara and Kawano, 1986a, b, 1987; Ohara and Utech, 1986, 1988), and molecular systematics (Kato et al., 1995a, b).

One of the most interesting findings of the present study is that topologies of the phylogenetic trees constructed by cladistic analyses of 22 gross morphological and one floral fragrance characters and molecular analyses of chloroplast DNA (RFLP analyses) (Kato et al.,1995a) showed a considerable similarity. As was expected, the genus consisted of two major groups in the molecular analyses, the pedicellate-flowered group and sessile-flowered group (Fig. 8 in Kato et al., 1995a; cf. Figs 1 and 2). Taxonomically, the genus has thus far been divided into two major subgroups, the pedicellate-flowered group (subgenus *Trillium*) and the sessile-flowered group (subgenus *Phyllantherum*) (Freeman, 1975), depending upon the presence or absence of the pedicel. In this respect, earlier classic taxonomic concepts of the infrageneric subgroupings (Freeman, 1975) are largely supported by the molecular studies.

However, a series of present studies have simultaneously brought forward some unexpected new findings. Both cladistic and molecular analyses revealed that pedicellate species within the genus *Trillium* did not form a distinct monophyletic group. In the molecular analysis, most species in the genus *Trillium* belong to one weakly supported clade (68% of the bootstrap replications), consisting of major members of the pedicellate-flowered species and all of the sessile-flowered species. It is noteworthy that in the molecular analysis *T. undulatum*, *T. rivale* and the above-mentioned large clade are sister groups, and the pedicellate-flowered species, *T. grandiflorum* and *T. ovatum*, are also paraphyletic to the whole sessile-flowered species, with many autoapomorphic site changes. In the cladistic analyses, *T. undulatum* and four other species also formed paraphyletically distinct clades, while *T. grandiflorum* and *T. ovatum* are paraphyletic to the major pedicellate group. In this respect, we can recognize a more or less congruence between the results of molecular analysis and cladistic analysis. However, *T. govanianum* and *T. taiwanense* formed a distinct clade, but are paraphyletic to the Sessile group in the cladistic analysis. We cannot interpret well at present whether or not this status reflects true affinities among the genus. Future molecular analysis is definitely needed, covering several other representative species that belong to different clades of the tree constructed by the cladistic analysis.

It is now very clear that sessile-flowered species form a distinct monophyletic group within the genus. This grouping was supported by both molecular and cladistic analyses. There is thus good support for recognizing subgenus *Phyllantherum* as a distinct subgrouping. Berg (1958) also suggested that the sessile-flowered species (subgenus *Phyllantherum*) are monophyletic in origin because of the fruit and pollen structure and other morphological similarities. Berg's (1958) interpretation was fully supported by the fact that there are few *cp*DNA variations among the sessile-flowered species in comparison with much higher variation noted among pedicellate-flowered species, indicating their higher affinities (Kato et al., 1995a).

Takahashi (1982) suggested that the sessile-flowered species are closely related to the pedicellate-flowered species, *T. erectum* and *T. cernuum*, based on his palynological studies. All these species have granulate pollen grains (with the exception of *T. petiolatum*, which has corrugate-type pollen). Our *cp*DNA data, however, indicated that the nearest relatives of the sessile-flowered species are the pedicellate-flowered species, *T. ovatum* and *T. grandiflorum*. These two species have spinulate pollen grains and many other

distinct micro-morphological and gross-morphological traits in common (Takahashi, 1982), e.g., exceptionally possessing "capsules" among the pedicellate-flowered species (Berg, 1958). However, palynological data of some other taxa of *Trillium* are not available at present, and thus we cannot further evaluate the significance of this particular trait in relation to the result obtained in the present cladistic analysis.

The molecular analysis showed that among pedicellate flowered species, *T. erectum, T. sulcatum, T. cernuum, T. rugelii* (all from eastern North America) and *T. kamtschaticum* (from East Asia) form a very well supported monophyletic clade (Kato et al., 1995a), and the cladistic analyses also fully support the Erectum group of the pedicellate species as a distinct clade, although the tree topologies obtained in these two different analyses are not exactly the same. However, mention should be made here that in the cladistic analyses (cf. Table 2), if reversal changes in the character states occur in some specific characters, the tree topology will be considerably different, and thus we have to evaluate the results with a certain reservation, even if a similar topology was obtained by different approaches (Fig. 2; Fig. 8 in Kato et al., 1995a). However, one conspicuous advantage of the cladistic analysis is that the results could be repeatedly tested by introducing different evaluations of character state and also additional characters. In any rate, we have to await future studies to confirm whether or not which trees are reflecting a true phylogenetic relationship among the species of *Trillium*.

Utech (1980) and Samejima and Samejima (1987) have suggested that pedicellate-flowered *Trillium* species in North America can be divided into two subgroups within subgenus *Trillium*; the Grandiflorum subgroup with about eight species including *T. grandiflorum, T. ovatum, T. undulatum* and *T. rivale*, and the Erectum subgroup with about six species including *T. erectum, T. sulcatum, T. cernuum* and *T. rugelii*. Both cladistic and *cp*DNA analyses supported the monophyly of the Erectum subgroup, but the results showed that the Grandiflorum subgroup *sensu* Utech (1980) is quite heterogeneous and does not form a simple single clade.

In the light of all available data at present, including the results of cladistic and molecular analyses (Kato et al., 1995a), the pedicellate-flowered group can be divided into at least two or three major clades, i.e., the Erectum group (*T. apetalon, T. smallii, T. kamtschaticum, T. tschonoskii, T. erectum, T. sulcatum, T. cernuum, T. flexipes, T. rugelii, T. vaseyi, T. catesbaei, T. grandiflorum,* and *T. ovatum*), the Govanianum group (*T. govanianum* and *T. taiwanense*), and three loosely grouped paraphyletic clades (i.e., *T. persistens; T. undulatum* and *T. rivale; T. nivale* and *T. pusillum*) to the major clades, the Erectum and Sessile groups. Indeed, these species referred to different clades or groups may

not be monophyletic in their origin, considering a tree topology obtained by the RFLP analysis of *cp*DNA (Kato et al., 1995a).

2. Evolutionary Changes in the Character States

Evaluation of the character states is very crucial in the cladistic analysis, and if our evaluation of characters—e.g., of whether or not a specific character is plesiomorphic or apomorphic—is not precise, the results obtained no doubt will be considerably biased by our erroneous character evaluation. In this respect, we always have to make some reservation concerning the results obtained by this method. On the other hand, if we can evaluate the characters in question in terms of evolutionary as well as ecological implications—that is, in relation to their roles or adaptive functions as life history traits—then we can interpret evolutionary meanings of changes in the character states more rigorously. Berg's studies (1958) are good examples. We must understand here that the consequences of evolutionary constraints, i.e., the interactions of phylogenetic and environmental constraints, are all manifested in the taxonomic characters used for diagnosis as well as life history traits of a given plant group (Kawano et al., 1992). This fact implies the significance of comparative approaches (Kawano, 1994).

As trachysporous mechanisms in *Trillium*, Berg (1958) cited the following three characters (cf. also Kawano et al., 1992): (i) declination of the stem and deposition of the mature fruit close to the ground shortly before its dehiscence due to the weak stem base, e. g. as found in *T. chloropetalum*, one of the West Coast sessile-flowered species; (ii) the presentation of the fruits close to the ground surface, facilitating seed dispersal and establishment, as found in *T. petiolatum*; and (iii) the extreme declination of the pedicel after anthesis, as found in *T. erectum* and *T. rivale*. All these characters—(i) the oil- and sugar-containing seed appendages (elaiosomes)(Berg, 1958; Nishino, Yamaoka, Ohara and Kawano, in preparation), (ii) the green capsules without sclerenchyma and a dehiscence mechanism, (iii) the green, persistent and enlarging calyx, (iv) the declining pedicel bearing a flower and berry, (v) the declining pedicel bearing a capsule, (vi) the development of coloration in berries and capsules, (vii) the weak overtuning stem, (viii) the presentation of a flower and fruit at the surface of the ground, (ix) offshoot formation at several portions along the rhizomes, and the release of fragrance or fetid odor at anthesis, attracting pollinators (Berg, 1958; Samejima and Samejima, 1987; Kawano et al., 1992; Kawano, 1994; Kawano et al., unpubl. data and obs.)—no doubt represent apomorphic character states with obvious adaptive significance. Loss of endozoochory—i.e., a change to myrmecochory (a change from the berries producing seed with pulpy sugar- and oil-containing elaiosome to

capsules producing seed with non-pulpy, only oil-containing seeds)—and loss of the pedicel are perhaps two major evolutionary changes in the character states with extraordinary adaptive significance in the evolutionary history of *Trillium* (Ohara and Higashi, 1987; Higashi et al., 1989; Kawano et al., 1992). However, we can also recognize some other evolutionary changes that have occurred in the character states of *Trillium*. These include the development of ornithochory in *T. undulatum*, which bears a brilliant scarlet red berry, although this may be a mimicry of the nutrient-rich fruits of other shrubby species containing seeds with a hard seed coat, since no pulpy sugar-rich carpel walls are formed in the fruit; furthermore, seeds with only oil-containing appendages (elaiosomes) are produced in *T. undulatum*, which is indeed a typical character of myrmecochory (Kawano et al., unpul.).

3.   The Adaptive Significance and Roles of the Pedicel
In *Trillium*, the pedicel has been regarded as very important both in terms of taxonomy and in its evolutionary-ecological function; it not only has diagnostic value for the recognition of taxa but also plays a significant biological role in attracting pollinators and/or seed dispersal agents in the pre- and post-reproductive stages. Obviously flowers with large open petals on the erect pedicel may attract a number of pollinators by visual signals. Indeed, a number of small Hymenoptera, Diptera, Coleoptera, etc. are known to be attracted to the white-flowered *Trillium* species (Ohara and Kawano, 1987; Kawano, 1994). The declining pedicel at anthesis, which occurs in several eastern North American declinate-flowering species (*T. catesbaei, T. cernuum, T. flexipes, T. rugelii*, and *T. vaseyii*), suggests a significant change in pollination and seed dispersal mechanisms. The change in the character state from the erect pedicel to declinate pedicel at anthesis suggests that there may be a major switching of pollinators from small flies, bees and beetles to large bumblebees such as *Bombus, Xylocopa, Lassioglossum*, and also some butterflies, since only these insects could forage for nectars in such downward- blooming or hanging flowers.

The declinate pedicel may, however, be a synapomorphic character, because such evolutionary changes in the character state may occur in parallel within the group (Fig. 3). However, the change of the character state from the erect to declinate pedicel during the fruiting stage is an entirely different story in terms of dispersal biology. We have often observed in the field that loss of mature fruits, especially those of *T. kamtschaticum* and *T. erectum*, etc., by predation of small mammals is very high. All marked mature fruits were lost by obvious animal predation within a couple of weeks after marking (Kawano, Utech and Ohara, unpubl. obs.). Thus, mature fruits borne beneath large foliage leaves may have a special role. Since a single

fruit of these species often contains one to three hundred seeds, successful dispersal of seeds from fruits that escaped mammalian predation damage would be very important sources for offspring recruitment (Ohara, 1989; Kawano et al., 1992).

Likewise, as noted earlier, the release of fragrance (e.g., *T. luteum*) or exceedingly strong unpleasant fetid odors (e.g., *T. foetidissimum*), possibly attracting small bees and dung flies, is another unique adaptive change occurring in the sessile-flowered group, accompanied by loss of the pedicel. Indeed, all these eastern sessile-flowered species bear dark-red, reddish purple or greenish purple petals, often enclosing both androecia and gynoecia in upwardly open petals (*T. lancifolium, T. recurvatum*, etc.), or completely exposing androecia and gynoecia to flower-visiting insects by having completely patent or somewhat ascending and twisted petals (*T. stamineum, T. decumbens*, etc.).

We now know that in Asia there occur two other very rare species, i.e., *T. taiwanense* of Taiwan and *T. govanianum* of the Himalayas. However, the extremely peculiar (and discontinuous from any other Asian or North American species) floral morphology of these two species is still inexplicable in terms of any adaptive significance, and must await more critical studies.

4.   Evolutionary Scenarios in Habitat Radiation and Character Differentiation of Trillium
Examination of geographical ranges and underlying environmental conditions within habitats, together with data on species' character states, provide extra information on the evolutionary-ecological status of the plant group under consideration (Kawano et al., 1992). The role of selection regimes which may have operated in the past and may have been responsible for the differentiation of important life history traits must, however, be likewise carefully assessed. The evolutionary scenario of a given plant group will then be revealed more clearly.

We now know that *Trillium* occurs in a wide and diverse range of habitats and environmental conditions. Judging from the present-day geographical ranges and habitats of *Trillium* species (Fig. 4), we can consider that the mesic forest floor and gentle seepage slopes of typical summer-green broad-leaved hardwood forests of the northern hemisphere, dominated by various oak, maple, and beech species (Shelford, 1963; Samejima and Samejima, 1987; Kawano et al., 1992), are the original habitats for the ancestral pedicellate-flowered species of *Trillium*. Most pedicellate and declinate *Trillium* species are primarily associated with this type of habitat.

Coniferous or mixed forests with deep shade, as found, e.g., in eastern North American (*T. undulatum, T. persistens*, etc.) and Himalayan (*T. govanianum*) habitats, seem to provide conditions for *Trillium* species that may have once been typical spring plants on

rich deciduous forest floor in spring. Photosynthesis is essentially a high-energy harvesting system, so that adaptation to deeply shaded environments with weak and diffuse light harvesting photochemical systems (Björkman and Ludlow, 1972; Kawano et al., 1992) appears, evolutionarily, to be a considerably time-consuming process. In this respect, the origins of these species must date back to the remote past, perhaps much further than the origins of some other groups of *Trillium* species. Likewise, several sessile-flowered species of western coastal coniferous forests (*T. angustipetalum*, and *T. kurabayashii*) as well as those of the southeastern North American Coastal Plain or eastern lowland mixed forests or upland rocky woods and on floodplains of streams (*T. foetidissimum, T. maculatum, T. sessile*, etc.) (Freeman, 1975) occupy a more or less similar environment with rich light resources (with many scattered sunflecks here and there) in spring today, but may have differentiated during inter- or even post-glacial periods of the Pleistocene Ice Age (Graham, 1972; Narin, 1964). *T. ovatum* of western North America exhibits a broad but somewhat similar ecological range, occurring under redwoods (*Sequoia*) as well as mixed overstory. *T. ovatum*, however, also occurs in somewhat arid conditions beneath Ponderosa and Lodgepole pine forests in the northern Rocky Mountains and the interior parts of the West Coast. These inland montane populations exhibit a much richer genetic variation than those of the Coastal Range populations, indicating their rather recent postglacial range extention (Fukuda and Channell, 1975).

Dark shady and damp river bank habitats covered by evergreen *Magnolia* and deciduous *Quercus* hardwood trees, and often exposed to flooding, notably in the Coastal Plain of eastern North America (Ohara and Utech, 1986, 1988), may represent another more recently derived habitat for *Trillium*. This is a typical habitat for most of the sessile-flowered species. A somewhat similar situation is found in the valley-bottom habitats of western North America, where *T. albidum* and *T. chloropetalum* occur. Berg (1958) described *Trillium* species in the floodplain habitats which tend to have very weak and fragile stems that readily fall to the ground surface when bearing mature fruits. In addition, those which grow in such unstable flood plain and riverine habitats have also developed a means to propagate effectively by offshoot formation. Although their fecundity is characteristically low, they bear larger, non-pulpy seeds (Ohara and Utech, 1986).

Unstable dry rocky habitats with good drainage, as found in *T. rivale*, developed on the marginal slopes of coniferous forests, and floodplains under alders etc., as well as xeric grassy rocky grasslands in scattered conifers of *T. petiolatum* extending over the eastern side of the Cascades and the Palouse grassland region in western North America, represent two extremes of the habitat expansion. Both species possess, interestingly

enough, long petioles, with most of the stem of *T. petiolatum* buried deep in the soil layer. There is no doubt that such a growth form is an adaptation to extremely dry summer and xeric habitats and to regional volcanic activity. The mature peduncle of *T. rivale* at the fruiting stage becomes exteedingly elongated, and fruits fall to the ground surface. This is obviously a mechanism to facilitate seed dispersal and establishment in the surroundings. These stem-peduncle modifications may be unique adaptive characters of these species to such extremely dry habitats, especially in summer.

5. Palaeoclimates and the Differentiations of Trillium

Within *Trillium*, the taxonomic ditribution of pedicellate-flowered species indicates a very ancient origin for this condition: all species in *Paris, Kinugasa* and *Daiswa* have pedicellate flowers, as do most species in *Trillium*. Our analysis of *cp*DNA variation did not provide a good resolution of phylogenetic relationships among all pedicellate species of *Trillium* (Kato et al., 1995a). Nonetheless, the analysis is consistent with the suggested ancient origin of the pedicellate condition. We therefore suppose that the genus *Trillium* was represented by pedicellate-flowered species during the Tertiary period, at least before late Tertiary or early Quarternary.

From the wide taxonomic distribution of the pedicellate condition, and the narrow taxonomic range of sessile-flowered species, Freeman (1975) argued that sessile-flowered species evolved from pedicellate species. The monophyly of sessile-flowered species was confirmed in our analysis of *cp*DNA variation (Kato et al., 1995a). The sessile-flowered group (subgenus *Phyllantherum*) may have evolved from pedicellate ancestors during the Tertiary period or more recently. The close affinities of the many living species within subgenus *Phyllantherum* suggest a relatively recent history of differentiation. At present, the subgenus *Phyllantherum* is composed of species that are all very similar in terms of gross morphology and *cp*DNA variation. The speciation of sessile-flowered species may have been promoted by frequent isolation and reunion of different populations during the alternation of glacial and interglacial periods. During the Quaternary period, beginning one million years ago, the Northern Hemisphere experienced progressive cooling, with a series of at least 23 glacial-interglacial cycles (van Donk, 1976). During glacial periods, vast areas of the North American and Eurasian continents were covered by ice (Delcourt and Delcourt, 1981, 1983, 1987).

From palaeoclimatological and palaeovegetational evidence, it is known that the vegetation was continuous across western and eastern parts of the North American continent 2.5–1.6 million years ago (Bowen, 1978; Boellstorff, 1978). During that period, many so-called temperate Arcto-Tertiary elements may have

had more or less continuous distributions across the North American continent. Some taxa may also have been distributed continuously from northeastern Asia to western and eastern North America. We believe that the subgroup Erectum was distributed continuously from East Asia to North America, once at least, some time during the late Pleistocene and possibly earlier. We cannot, however, judge in absolute terms the age of this subgroup. Future more ctitical studies no doubt will shed light upon the evolutionary history of this most interesting group of the Trilliaceae, the genus *Trillium*.

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