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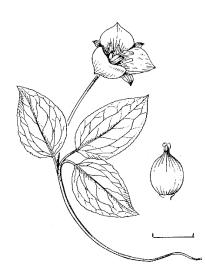
What can DNA molecules tell us about Trillium?

by Kenton L. Chambers

In Oregon, the genus *Trillium* is only moderately diverse, with 5, or at the most 6, species, depending on whether one treats the northern Willamette Valley forms of *T. albidum* as a separate species or not. However, in the southeastern United States, it becomes extremely variable, and *Flora of North America* (Vol. 26) describes 17 species from that region, quite a few of which are said to form interspecies hybrids. Like so many lily family members, trilliums are uniquely attractive to many people, and certain species can be grown in the garden from their long-lived rhizomes. In the interests of conservation, unthinking harvesting of plants for this purpose is to be strongly discouraged!

There has been a wholesale redefinition of family units *within* the classical Liliaceae, with a maximum of 27 segregate families now being recognized for North America (Utech 2002). In the remodeled Liliaceae itself, the only remaining large native genera are *Lilium*, *Fritillaria*, and *Erythronium*. In the new family Trillia-

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Trillium (Pseudotrillium) rivale. Scale=2cm. Courtesy of Stanford University Press, Illustrated Flora of the Pacific States, Vol. 1. Illustrated by W.S. Atkinson.

Your contributions bring the *Flora of Oregon* closer to completion

by Linda Hardison Oregon Flora Project Coordinator

Our 2005-2006 fundraising campaign has been different from past efforts. While our prior fundraisers have been initiated by a large contribution as a challenge, this fund drive has been a simple appeal to support our worthy cause. And the support has been outstanding! From the date of the initial appeal letter, 30 November 2005, until 15 May 2006, we have received \$55,248—thank you very much! This reflects a very generous gift from an anonymous donor, 308 donations from individuals and small businesses, three NPSO chapters, and the state NPSO. We are also happy to recognize 48 first-time donors in this campaign.

As a comparison, the 10th Anniversary Challenge Drive (November 2005 – January 2006), raised a total of \$65,597. During the 2005 calendar year, individuals and NPSO entities contributed \$73,147; and thus far in 2006, we have received \$34,087. This year we have been awarded \$25,500 in new grants, and have submitted requests for \$30,000 in proposals that are now in the review process. However, as explained elsewhere in the newsletter, an earlier grant and the donations which were used to support our illustrations editor ran out in January.

How will the Oregon Flora Project sustain its activities for the remainder of the year, and in the future? To put this question in context, our annual budget will average in excess of \$138,000. This reflects the reinstatement of a full-time illustrations editor, and the expense of 100% of our database managers' salaries that ensues with the July 2006 completion of the databasing grant held jointly with the Herbarium. As the value of OFP resources are appreciated and utilized by a growing number of users, we request that these individuals, businesses, and agencies join our circle of financial supporters. Grant proposals to both government agencies and foundations will continue to be developed and submitted. We encourage other NPSO chapters to follow

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ceae, *Trillium* is joined by three Eurasian genera, *Paris*, *Kinugasa*, and *Daiswa*. Approximately seven *Trillium* species and 3 named hybrids are native to eastern Asia, including the Himalayas.

With the above introduction, we can address a recently published phylogenetic study of Trilliaceae based on a combination of morphological and molecular data (Farmer and Schilling 2002). This study draws some significant conclusions about one Oregon species in particular—*Trillium rivale*—which appears to stand apart from all other trilliums. As discussed below, the molecular cladistic technique used by Farmer and Schilling may have contributed to their decision to describe the new genus *Pseudotrillium* for this species.

We know *Trillium rivale* as a characteristic plant of stream-banks in serpentine soil areas of southwestern Oregon, often associated with *Darlingtonia* bogs and seeps. According to Farmer and Schilling, the morphological features setting the species apart from other trilliums are (1) its purple-spotted petals, (2) its petiolate leaves that are "thick, leathery, (and) cordate," and (3) its floral pedicels that elongate after anthesis, bringing the fruit in contact with the ground. Its spotted petals, they tell us, are unique in the genus *Trillium*.

I made a study of the 49 sheets of *T. rivale* in the herbaria at Oregon State University (OSC, ORE,

Erythronium oregonum logo and masthead designed by Tanya Harvey.

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WILLU). Of these, 9 sheets have plants with somewhat cordate cauline leaves, 18.4%; the rest have truncate or rounded leaf bases. Mounted on the sheets are 110 flowering individuals. Petal spotting is variable, ranging from none to faint to well-marked. Petals of older collections may have lost their faint spotting patterns. Counting these as non-spotted, however, along with plants having undoubtedly plain, unspotted petals, I found 57 plain and 53 spotted-petal individuals, a ratio of 52% to 48%. The leaves on all these pressed specimens have the same thin texture as other Trillium collections, with no sign of having been thick or leathery. (Perhaps Farmer and Schilling are referring to the condition mentioned by Case (2002), who states "(a)fter flowering, the entire plant may enlarge and become more robust and turgid with very glossy leaves." However, post-flowering changes like this need to be studied and described for all Trillium species, not just *T. rivale*.)

Downward curvature of the floral pedicel in fruit also occurs in other *Trillium* species, as part of the genus' seed-dispersal adaptations. The pendent capsules split open to drop the seeds, whose attached food-body is attractive to ground-dwelling ants (Berg 1958). Thus, the three described features of "*Pseudotrillium*" are hardly so unique as to justify a generic separation of *T. rivale*.

The paper's molecular phylogeny involved DNA sequence comparisons of two genes, *matK* and ITS, which are commonly used in plant systematics. Sequences for 26 species of *Trillium, Paris*, and the other genera were available. The nucleotide sequence for the ITS gene(s) of *T. rivale* has 5 unique changes (called "indels"—insertions-deletions) compared with the 25 other species. A total of 25 nucleotides have been inserted into, or deleted from, the ancestral gene(s) in the evolution of this species. When these differences were factored into the molecular-cladistic analysis, along with other sequence evolution in the 26 species, *T. rivale* was diagnosed as being separate and basal to all the rest of the group—according to the cladistics programs used by the authors.

In cladistic theory, a reason for giving generic status to *Pseudotrillium rivale* is that otherwise, if it remains within *Trillium*, that genus becomes "paraphyletic*" to *Paris, Daiswa*, and *Kinugasa*—and paraphyletic genera are forbidden in cladistic taxonomy. An alternative taxonomy would be to enlarge *Trillium* by including in it the three Asian genera, eliminating the separation of *Pseudotrillium*. Evidence for a close relationship of all these genera is provided by studies of morphology, embryology, chromosome morphology, pollen, and molecular *rbcL* gene phylogeny (Kazempour and Kawano 1999). If this group is an ancient one, with extinct "missing links" between the ancestors and modern species, then "we cannot precisely evaluate the phylogenetic

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positions of these different taxa based only upon the molecular data available at present" (Kazempour and Kawano 1999).

The puzzle remains, as to whether we should make the leap of faith that molecules have the final say, or stay with the conservative view that *Trillium rivale* lacks enough unique morphological differences for it to be removed from *Trillium*. We may lean towards *Pseudotrillium* in the future, but for now, I am reluctant to change the generic placement of this species "based only upon the molecular data available at present."

*Paraphyletic, in the sense used here, means that if *Trillium* continues to contain *T. rivale*, the genus would not include all of the descendents of the common ancestor of this phylogenetic group or "clade." A "monophyletic" genus *Trillium*, which cladistic theory demands, must exclude *Paris*, *Kinugasa*, *Daiswa*, and *Pseudotrillium rivale*. If all 4 genera were united into *Trillium*, however, that genus would then be monophyletic. This approach is favored, in fact, by amino acid sequencing of the protein coded by the *matK* gene (Kazempour and Kawano 1999), showing that the issue is not settled.

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Kazempour Osaloo, S. and S. Kawano. 1999. Molecular systematics of Trilliaceae. II. Phylogenetic analyses of *Trillium* and its allies using sequences of *rbcL* and *matK* genes of cpDNA and internal transcribed spacers of 18S—26S nrDNA. Plant Sp. Biol. 14: 75-94.

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the example of Emerald Chapter by initiating matching challenges among their members. And, as we strive to maintain data accessibility for all users, we must also raise public awareness that the botanical information provided by the Oregon Flora Project requires financial resources to research, curate, and upgrade.

Whether your support of the OFP involves contributions of your time and expertise, your dollars, or both, be assured it is greatly appreciated! It is our shared vision of the value of this botanical work that will help propel us to a new *Flora of Oregon*. Thank you!

The Oregon Flora Project's Photo Gallery: a spring bouquet

by Rhoda Love

In February we asked members of NPSO to name their favorite spring wildflowers as a way to give our readers a preview of the Oregon Flora Photo Gallery. Soon images such as these will be available to all computer users as part of our Project's comprehensive coverage of the flora of Oregon. The images here can be enjoyed in full color on the web version of the *Oregon Flora Newsletter* at www.oregonflora.org.



Photo: Tom Kaye

Western trillium (Liliaceae: *Trillium ovatum*), and bleeding heart (Fumariaceae: *Dicentra formosa*) are the favorite early blooms of Les Helgeson of Tillamook on the Oregon coast-- especially, he writes, when they occur together as they often do in mid-March.



Photo: Linda Hardison 'possible cultivar').

Leila deLaubenfels loves the elusive and beautiful annual tricolor monkeyflower (Scrophulariaceae: *Mimulus tricolor*), which she photographed on her family farm near Corvallis.



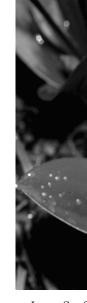
Photo: Leila deLaubenfels



Snow queen (Scrophulariaceae: *Synthyris reniformis*) is a favorite of John Savage, Salem.



Don Jacobson of Portland wished readers a "Happy spring," when he found the yellow woodland violet (Violaceae: *Viola glabella*) in bloom at Tryon State Park on February 12.



Larry Scof spring flower (Liliaceae: *Sc* spring in the amute River.



Dave Wagner of Eugene searched for open flowers of osoberry (Rosaceae: *Oemleria cerasiformis*) near Eugene and reported, "They're out," on February 4th.



Dorothy and Ted Schattenkerk of the Umpqua Valley were excited to find Umpqua mariposa lily (Liliaceae: *Calochortus umpquaensis*) at Little River.



orite of do Leo-finds it

Whitlow grass, (Brassicaceae: *Draba verna*), is a favorite of Jerry Baker of Pendleton who included this quote from Aldo Leopold, "He who searches for spring on his knees in the mud finds it in abundance."



ield of Salem writes that his favorite is the unique fetid adder's tongue oliopus hallii), which he finds, each Coast Range near the Little Lucki-



Dave Predeek of Eugene loves hunting for the diminutive rare white dwarf rue anemone (Ranunculaceae: *Enemion stipitatum*) along the Willamette River flood plain in February.



Photo: Jean Waller

Cindy Roché of Medford considers Henderson's fawn lily (Liliaceae: *Erythronium hendersonii*) "one of the wonders of the world."



Yellow bells (Liliaceae: *Fritillaria pudica*) in the Columbia Gorge are a spring favorite of Paul Slichter.



Veva Stansell of Pistol River is thrilled each January and February to find a few blooms of Howell's saxifrage (Saxifragaceae: *Saxifraga howellii*) on wet rocks along the north bank of the river.

We are sorry that space did not allow us to use all the suggestions we received. In addition to those above, Karl Anderson mentioned *Sidalcea malviflora*, and Barbara Robinson noted *Lomatium piperi*. Many thanks to everyone who wrote to nominate their favorite spring blooms.

Project news: new features coming your way!

by Linda Hardison

Our feature article showcasing spring wildflowers is illustrated with photographs from the Oregon Flora Photo Gallery; you may also view these photos in full color in the online version of the newsletter (www.oregon-flora.org). These are but a sampling of the thousands of images in our OFP files. We continue to work with our partner NACSE (Northwest Alliance for Computational Science and Engineering) to prepare the pictures for release as part of the Digital Field Guide. If you enjoy photographing plants, please consider contributing your pictures for inclusion in our Photo Gallery. You will find general instructions and lists of taxa not currently part of our collection on our website: www.oregonflora.org/oregonphotogallery.html.

With respect to our newsletter, we have recently converted all the archival issues of the Oregon Flora Newsletter to pdf files. This format will make it easier for you to retrieve a complete issue of the newsletter, and where possible, will provide images in color—yet another way you can enjoy the photographic resources of the Oregon Flora Project! Articles are searchable, and are indexed by author and issue.

We are very pleased to announce that a \$10,000 proposal to complete the Poaceae (Grass Family) component of our morphology database was recently funded by Oregon State University's General Research Fund. The research accomplished will be a building block that supports further development of the multiple-entry key and the floristic descriptions for the grasses. It also represents the first direct support from the University in the twelve years of the project's existence.

June will mark a milestone in the development of the Oregon Flora Checklist. We will be sending to the Checklist Advisory Board and Checklist Project Leaders the proposed changes completed by our manuscript authors. With feedback from the advisory groups (see names, page 2), we will incorporate approved changes into the Checklist. This summer you can look forward to a public release of a draft of the Oregon Vascular Plant Checklist, containing accepted names and synonyms of all taxa known to occur in our state. You will be able to find the Checklist on our website in a format you can download and print at home.

The electronic Atlas, which many of you have been using, will undergo a data upload this summer that will add over 30,000 new mappable plant records. Incorporating new data, assuring its quality, and expressing it in the context of the Atlas are tasks that are necessary to sustain this resource and keep it as up-to-date as possible. Your financial support allows us to do this essential work and keep the Atlas both functioning and

expanding. You will want to view our updated maps once the new data have been added.

Since 2003, our Illustrations Editor, Rena Schlachter, has been an OFP staff member whose creativity and skills have contributed to many aspects of the Project, especially those that inform the public about our mission. Rena has been our webmaster, the wizard who produces this newsletter, and manager of our images databases. Unfortunately, with the completion of the grant that supported her position, and our recent dearth of funding, Rena's full-time position was reduced to one day a week as of January this year. Financial reality has further prevailed, and we sadly acknowledge that Rena will be leaving us at the end of June for a full-time position as project manager for a graphic arts firm in Portland. We send Rena our very best wishes for success at her new undertaking, and we look forward to seeing her illustrations in the new Flora of Oregon as our future funding and her time allow!

Thanks

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How can I contribute?

Donations to the Oregon Flora Project are a critical part of our operating budget. Your contributions help pay the salaries of our staff and students, as well as all newsletter expenses.

There are two ways to donate to the Oregon Flora Project: (1) with a check payable to the Oregon State University Foundation, attn: Oregon Flora Project; and (2) through the Friends of the Oregon Flora Project, with a check payable to the Native Plant Society of Oregon, attn: OFP.

With your contribution, please let us know if you do not wish your name listed in our "Thanks" column, and if you would like to be added to our Oregon Flora Newsletter mailing list.

Mail your check to:

Oregon Flora Project P.O. Box 402 Corvallis, OR 97331-2902

Volunteer position available

The Oregon Flora Project is seeking a volunteer to assist with project correspondence, bookkeeping, and administrative activities. Basic word processing, familiarity with Internet navigation, and written communication skills are needed. Flexibility in scheduling is assured, but willingness to commit to 2 hours per week for a 6 month (or longer) duration is needed. Most of the work is to be performed on the OSU campus. Please contact Linda at (541) 737-4338 or hardisol@science.oregonstate.edu for more information. This is an opportunity for an individual to apply and expand their lifeskills while making a much-needed and meaningful contribution to the development of the Flora of Oregon.



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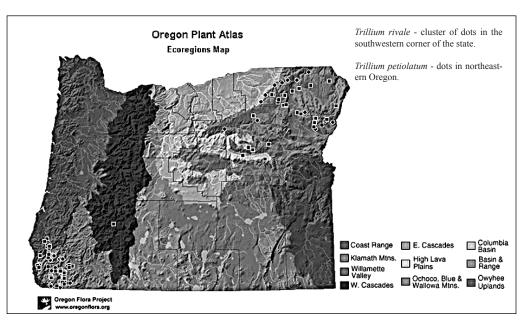


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Did you know?

- The Oregon Checklist treatment of *Trillium*, authored by Dr. Kenton Chambers, currently recognizes five species in the state: *T. albidum*, *T. kurabayashii*, *T. ovatum* ssp. *ovatum*, *T. petiolatum*, and *T. rivale*.
- The Oregon counties with the greatest *Trillium* diversity are Curry (*kurabayashii*, *ovatum*, and *rivale*), Douglas, and Josephine (both with species *albidum*, *ovatum*, and *rivale*).
- Seven counties lack any reported occurrences of this genus: Morrow, Gilliam, Sherman, Crook, Lake, Harney, and Malheur Counties.
- Among the OSU Herbarium specimens mapped in the Atlas, the *Trillium* most collected is *T. ovatum* ssp. *ovatum*, with 135 specimens.
- The greatest number of trilliums in the Herbarium have been collected during the month of April (116 specimens), with May (62), March (45), and June (41) being distant runners-up.



Trillium rivale, in the southwestern corner of the state, and *T. petiolatum* in northeastern Oregon represent two species of this genus that are distinct in their distribution and the ecoregions they occupy. The solitary incidence of *T. rivale* that maps to Black Rock Lookout in Douglas County, collected in 1944, was confirmed by Dr. Chambers as correctly identified but, he adds, the locality needs to be verified due to a possible error in labelling of the specimen.