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REPRODUCTIVE STRATEGIES AND BARRIERS TO HYBRIDIZATION BETWEEN *TELLIMA GRANDIFLORA* AND *TOLMEIA MENZIESII* (SAXIFRAGACEAE)[1]

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We examined the reproductive biology of *Tolmeia menziesii* and *Tellima grandiflora* (Saxifragaceae) and barriers to hybridization between these species. Crossing experiments and pollinator observations suggest that *Tolmeia* is completely self-incompatible, obligately outcrossing, and bumble bee pollinated, whereas *Tellima* is self-compatible, with a mixed mating system possibly facilitated by rove beetles. Hybrid crosses set seed only when *Tellima* was the pollen recipient. Observations of pollen–style interactions indicate that self-incompatibility in *Tolmeia* is late-acting and that pollen tube growth is comparable between reciprocal hybrid pollinations. Seven of 40 progeny from *Tolmeia* × *Tellima* crosses were isozymically and morphologically intermediate between *Tolmeia* and *Tellima,* and were identical to a naturally occurring hybrid. The remainder were identical to *Tellima,* the maternal parent in successful hybrid crosses, which we attribute to either intraspecific pollen contamination, or agamospermy. Barriers to hybridization between sympatric *Tellima* and *Tolmeia* consist of divergence in floral morphology, pollinator relationships, compatibility, and a series of postzygotic isolating mechanisms including hybrid sterility.

Key words: insect pollination; intergeneric hybridization; late-acting self-incompatibility; plant mating systems; reproductive isolating mechanisms; Saxifragaceae; *Tellima*; *Tolmeia.*

Reproductive isolating mechanisms traditionally have been invoked to explain the coexistence of related species (Grant, 1981). The occurrence of natural hybridization between species demonstrates that reproductive isolation is often incomplete, even at the generic level. For example, hybridization has been reported between *Besseya* and *Synthyris* (Kruckeberg and Hedglin, 1963), *Fragaria* and *Potentilla* (Asker, 1971), *Aciphylla* and *Gingidia* (Webb and Druce, 1984), and *Acaena* and *Margyricarpus* (Crawford et al., 1993). The scattered geographic distribution of rare natural hybrids between some Saxifragaceae suggests that barriers to hybridization are weakly developed in this family, especially among members of the *Heuchera* group (sensu Soltis et al., 1991a). Natural intergeneric hybrids include *Conimitella* × *Mitella* (Soltis and Soltis, 1986), *Heuchera* × *Tiarella* (Spongberg, 1972), *Mitella* × *Tiarella* (Spongberg, 1972), and *Tellima* × *Tolmeia* (Soltis and Bohm, 1985). Additionally, a hypothetical case of hybridization between ancestral species of *Mitella* and *Tellima* has been proposed to explain patterns of chloroplast DNA variation (Soltis et al., 1991a, b). Despite extensive systematic work on the Saxifragaceae sensu stricto, and numerous cases of natural hybrids, there have been no reproductive studies that examine hybridization in the group. In this paper, we present the most detailed study to date on hybridization between *Tolmeia menziesii* Pursh and *Tellima grandiflora* Pursh. We describe the nature of reproductive isolation between these species as an important context in which to understand their evolutionary divergence, and the maintenance of differences between them.

Tolmeia and *Tellima* overlap extensively in geographic range, from northern California to southern Alaska, and sympatric populations occur throughout (Hitchcock and Cronquist, 1973). Soltis and Bohm (1985) showed that natural *Tolmeia* × *Tellima* hybrids were sterile and shared the distinctive chromosomal features and flavonoid constituents of *Tellima* and *Tolmeia.* Hybrids collected in the southern and northern parts of the range had chromosome numbers $2n = 14$ and $2n = 21$, respectively, consistent with *Tolmeia's* geographically distributed diploid ($2n = 14$) and tetraploid ($2n = 28$) cytotypes. Confirmation of *Tolmeia* × *Tellima* was further supported by evidence from ribosomal RNA genes (Doyle, Soltis, and Soltis, 1985). The natural occurrence of hybrids in the Saxifragaceae led Soltis and Bohm (1985) to speculate that reproductive isolating mechanisms are not well developed in the family. Few ecological data are available to account for the absence of introgression between these morphologically distinct species with similar geographical range and habitat requirements (Kearns, 1983).

Savile (1975) hypothesized that differences in flower and fruit morphology between *Tolmeia* and *Tellima* reflect different pollination and seed dispersal mechanisms. Here we examine how morphological and mating system divergence influences the reproductive isolation of sympatric populations. We suggest that floral divergence between these taxa represents the evolution of different sexual strategies. The reproductive ecology of *Tolmeia* and *Tellima* is described in terms of pollination mechanisms,

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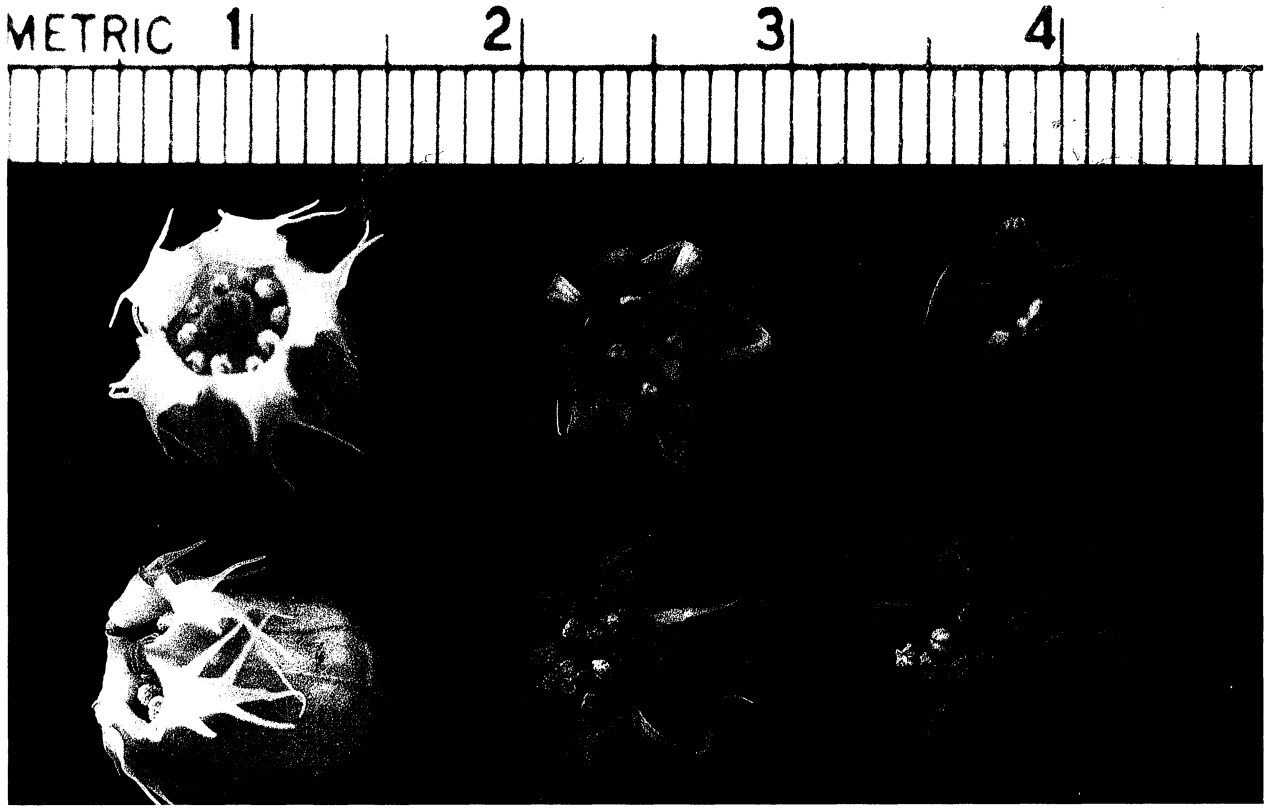


Fig. 1. Floral morphology of *Tellima grandiflora* (left), *Tolmeia* × *Tellima* (center), and *Tolmeia menziesii* (right). Scale in cm.

pollen–pistil interactions, and isozyme variation. We include observations on habitat, floral morphology and phenology, and pollinator visitation. In natural populations, hand-pollination experiments examined (1) the extent of self-incompatibility, (2) the effect of outcrossing distance on seed set, (3) the possibility of pollinator limitation of reproductive output, (4) the direction of hybridization, and (5) postpollination barriers to hybridization. Since hybrid crosses did not exclude alternative sources of parentage (e.g., pollen contamination and agamospermy), we also show that some progeny from intergeneric hand-pollinations share the morphological and allozymic characters that differentiate the parental species.

MATERIALS AND METHODS

Taxonomic status—*Tellima* and *Tolmeia* have similar vegetative morphology but striking differences in floral structure led to their classification as allied monotypic genera (Hitchcock and Cronquist 1973; Fig. 1). More recent taxonomic studies have employed chromosomal, isozyme, flavonoid, cpDNA sequence, and restriction site variation (Soltis, 1980, 1982, 1984b; Soltis and Bohm, 1985; Rieseberg and Soltis, 1987; Soltis et al., 1990, 1991b; Soltis, Soltis, and Ness, 1990). These authors note that the phylogenetic reconstruction of the Saxifragaceae is complicated by the questionable status of monotypic genera and the occurrence of intergeneric hybridization.

Tellima is diploid ($2n = 14$) throughout its range, but *Tolmeia* is diploid ($2n = 14$) and tetraploid ($2n = 28$) in the southern and northern portions of its range, respectively (Soltis, 1984a). An autopolyploid origin was proposed for tetraploid *Tolmeia* based on the morphological, flavonoid, and allozymic similarity of diploid and tetraploid cytotypes (Soltis, 1984a; Soltis and Rieseberg, 1986; Soltis and Soltis, 1988). Isozyme patterns suggest that tetraploid *Tolmeia* demonstrates tetrasomic inheritance and is more heterozygous than diploid *Tolmeia* (Soltis and Soltis, 1988, 1989). For example, *Tolmeia* tetraploids frequently show three or four alleles at a single enzyme locus (Soltis and Rieseberg, 1986). The degree of differentiation among chloroplast genomes of diploid and tetraploid *Tolmeia* suggests that autopolyploidy and subsequent reproductive isolation of *Tolmeia* cytotypes are ancient, with considerable cpDNA sequence divergence since that time (Soltis et al., 1989). It follows that the divergence of *Tolmeia* and *Tellima* lineages predates this event.

Floral morphology—*Tellima* and *Tolmeia* are perennial herbs with elongate many-flowered racemes arising from basal leaves (Hitchcock and Cronquist, 1973). The several racemes per plant have 10–45 flowers. Leaves and pedicels are hirsute, and the leaves are cordate-ovate, serrate, and shallowly lobed. *Tellima* is rhizomatous, forming clumps of interconnected plantlets (Kearns, 1983). *Tolmeia* also reproduces asexually by producing distinctive plantlets at the bases of the older leaves. *Tellima* has pentamerous flowers, composed of triangular sepals and pinnately-divided petals, with ten stamens arising just below the tepals (Fig. 1). The bicarpellate gynoecium has a partially inferior ovary with many ovules. Two thick styles taper toward the stigma, which is capitate and lobed. Following pollination, the carpels reflex and split longitudinally, exposing the ovules. The many seeds per fruit are brown, ellipsoid, and covered with longitudinal ridges. *Tolmeia* has a zygomorphic, tubular flower, easily distinguished from the actinomorphic flower of *Tellima* (Fig. 1). The hypanthium consists of three large and two small lobes, divided almost to the base with four recurved entire petals attached between lobes. Three unequal stamens (two long and one short) are opposite the larger lobes. The bicarpellate gynoecium has

TABLE 1. Measures of floral morphology in *Tellima grandiflora* and *Tolmeia menziesii* (Saxifragaceae) taken from flowers on separate plants. Pollen viability was assessed using Alexander's stain with 2% lactic acid (Alexander, 1980). The sugar concentration of nectar pooled from 25 flowers was measured using a vapor pressure osmometer.

	Tellima grandiflora			*Tolmeia menziesii*		
	$\bar{X} \pm$ SD	Range	*N*	$\bar{X} \pm$ SD	Range	*N*
Style length (mm)	4.9 ± 0.3	4.3–5.5	10	4.7 ± 2.2	1.6–7.8	10
Filament length (mm)	1.6 ± 0.2	1.3–1.8	10	3.8 ± 1.5	2.2–7.5	10
Anther length (mm)	0.4 ± 0.5	0.5–0.9	10	1.5 ± 0.3	1.0–1.8	10
Hypanthium width (mm)	7.0 ± 0.4	0.1–6.1	10	2.5 ± 0.3	2.1–3.3	10
Hypanthium length (mm)	11.9 ± 0.9	10.4–13.3	10	8.5 ± 0.7	7.3–9.6	10
Pollen grains/anther	4 300 ± 400	3 900–4 900	5	7 600 ± 1 400	5 700–9 200	5
Ovules/ovary	394 ± 93	234–568	16	100 ± 24	78–141	6
Pollen/ovule ratio	109	—	—	228	—	—
Pollen viability (%)	100	—	5	98	93–100	5
Nectar volume (10^{-1} μL)	4.1 ± 5.0	0–27.0	25	5.4 ± 4.8	0–25.0	25
Sugar conc. (mol/L)	0.44	—	1	0.80	—	1

a superior ovary, divided into two beak-like portions containing many ovules. During maturation, the styles recurve and the ovary splits ventrally to expose the developing seeds, which are black, round, and covered with minute spines.

Field site and habitat—We studied natural populations of *Tellima* and *Tolmeia* near the Sandy river, at The Nature Conservancy's Diack Tract and adjacent Oxbow County Park (Multnomah County, OR). Vegetation at the site is typical of the western Cascades *Tsuga heterophylla* zone (Franklin and Dyrness, 1988). *Tellima* and *Tolmeia* are locally common in secondary forest composed of red alder (*Alnus rubra*), big leaf maple (*Acer macrophyllum*), and Douglas-fir (*Pseudotsuga menziesii*). *Tellima* is an aggressive colonizer of exposures on disturbed slopes. *Tolmeia* occurs patchily in floodplains, streamsides, and roadside drainages. Despite subtle differences in microhabitat, sympatric populations occur near streams where the edaphic conditions appear to be favorable for both species. Floral measurements and pollinator observations were made in populations of *Tellima* and *Tolmeia* during May and June 1992. Morphological measures of flowers from separate plants are summarized in Table 1. We observed flower patches for insect visitors over several 60-min periods during the peak of diurnal insect activity (1000-1700), totaling 12 h of observation per species.

Hand-pollination experiments—Hand-pollinations were performed in the field on 28 *Tolmeia* and 39 *Tellima* plants, each bearing several racemes. A single raceme was pruned to eight floral buds that were emasculated and bagged with nylon netting. Within 1–2 wk of emasculation, an array of controlled pollinations was carried out: self, outcross, and hybrid. Other inflorescences on the plant supplied self pollen for emasculated flowers. In the outcross treatment, pollen collected at varying distances was applied to different recipients (1, 10, 100, and 1 000 m). Hybrid crosses were made reciprocally. As a control, a quarter of the emasculated flowers were not hand-pollinated. The four treatments were randomly assigned to individual flowers within each raceme. We used segments of monofilament line to transfer pollen from donor flowers to the stigmatic surface (770 ± 160 grains per application; $N = 3$). Additionally, the degree of selfing in the absence of pollinators was estimated by bagging unemasculated racemes. Natural levels of seed production were measured from tagged racemes.

A subset of the flowers was harvested at 24 h after hand-pollination and fixed in FAA (formalin: acetic acid: ethanol, 1:3:7, by volume) for microscopy. Pollen tube growth was visualized under UV-light using aniline blue (Ramming and Hinrichs, 1973). Gynoecia were immersed in 1.0 mol/L NaOH for 1 h to soften the tissue, rinsed and immersed in 0.1% aniline blue in 1 mol/L Na_3PO_4 for 3–5 h, and destained overnight in water. Pollen tubes were observed with a Leitz Laborlux microscope equipped with mercury vapor lamp (450–490 nm excitation filter and 530 nm barrier filter). We scored pollen tubes at four positions along the gynoecium (stigma, upper style, lower style, and base of the ovary), using an ordinal scale corresponding to the log of pollen tube number (i.e., 0, 1, 10, 100, 1 000).

Seeds from 90 of the 229 fruits resulting from hand-pollination were tested for germinability. After germination, seedlings were propagated in a greenhouse to insure that multiple individuals from each fruit were available for isozyme analysis. Isozyme variation was examined in 115 F1 progeny by starch gel electrophoresis.

Isozyme analysis—Isozyme variation is routinely used to estimate plant mating systems and population differentiation (Hamrick, 1989). Isozymes are also useful for studies of hybridization since F1 hybrids are expected to complement the allozymes of the two parent species. Examples of such hybridization studies include *Stephanomeria* (Gallez and Gottlieb, 1982), *Conimitella* and *Mitella* (Soltis and Soltis, 1986) and *Iris,* (Nason, Ellstrand, and Arnold, 1992). If the parental species in a hybrid cross show allozymic differentiation, then F1 hybrids can be identified based on their heterozygosity at that locus. We tested hybrid purity by examining allozymic variation at several loci that were previously shown to distinguish *Tolmeia* and *Tellima* (Reiseberg and Soltis, 1987).

Proceedures for electrophoresis followed Soltis et al. (1983). Enzymes were extracted from leaf samples in a modified grinding buffer: 0.010 mol/L KCl, 0.021 mol/L $MgCl_2$, 0.001 mol/L EDTA (tetrazolium salt), 12% polyvinylpyrolidone (40 000 molecular mass), 0.1 mol/L Tris-HCl (pH 7.5), and one drop mercaptoethanol (D. Soltis, personal communication). Two gel and electrode buffer systems were employed simultaneously to resolve the following five enzymes: leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), and shikimic acid dehydrogenase (SkDH). Gel staining and the interpretation of banding patterns followed Soltis et al. (1983).

RESULTS

Pollinator observation—Bumble bees were the primary insect visitors to *Tolmeia* flowers (Table 2). *Bombus edwardsii* Cresson was the most frequent visitor, although *Bombus flavifrons* Cresson, a flower fly (*Syrphus* sp.), and a solitary bee (*Halictus* sp.) were also observed collecting pollen from *Tolmeia* flowers. Foragers of *B. edwardsii* carrying *Tolmeia* pollen in their corbiculae typically visited one or two flowers per inflorescence, often "skipping" between several inflorescence patches. Bumble bees (*Bombus californicus* Smith and *B. edwardsii*) were rarely observed at *Tellima* flowers, which were often occupied by rove beetles (*Eusphalerum* sp.). Miller

TABLE 2. Insect visitors to flowers of *T. grandiflora* and *T. menziesii* were observed over 1-h intervals for a total of 12 h per species during May and June at the Sandy River Gorge, Oregon. Total observations of bee and fly visitors are listed, along with their relative frequencies and the mean number of floral visits per inflorescence. In the case of *Eusphalerum* beetles on *Tellima* flowers, frequency was estimated as the number of beetle-containing flowers in the sample.

	Tellima grandiflora			*Tolmeia menziesii*		
	Obs.	Freq.	$\bar{X} \pm$ SD (*N*)	Obs.	Freq.	$\bar{X} \pm$ SD (*N*)
Bombus edwardsii	2	0.08	4.1 ± 2.1 (6)	25	0.81	1.7 ± 1 (140)
B. flavifrons	—	—	—	2	0.06	1.6 ± 0.5 (5)
B. californicus	2	0.08	2.2 ± 0.9 (4)	—	—	—
Halictus sp.	—	—	—	1	0.03	1 (1)
Andrena sp.	1	0.04	3 (1)	—	—	—
Syrphus sp.	—	—	—	2	0.06	1 (2)
Eusphalerum sp.	21	0.80	3.2 ± 1.9[a]	—	—	—

[a] Beetles per flower.

(1985) reported the association of *E. pothos* with *Tellima* populations in the southern part of its range. At the Sandy River Gorge, we did not observe any actual movements of these small insects between inflorescences. Rove beetles and bumble bees are behaviorally very different, and thus patterns of floral visitation are difficult to compare. We counted the number of beetles per *Tellima* flower from a sample of 21 flowers from separate plants. Each beetle-containing flower was treated as an independent observation in our calculation of visitor frequency (Table 2).

Pollination treatments—Table 3 summarizies multiway ANOVA comparisons of seed set per fruit between pollination treatments, plants, and flower positions in *Tellima* and *Tolmeia.* All pollinations were included in the analysis, including flowers that did not set fruit. Variation in seed set among the pollination treatments exceeded variation within the treatments. In *Tolmeia,* pollination treatment and pollen recipient accounted for significant components of variation in seed set, but pollination treatment explained the greatest portion of the total variance. In *Tellima,* a significant component of variation in seed set was explained by pollination treatment but not by pollen recipient or flower position.

Table 4 summarizies pairwise comparisons of fruit and seed set among the pollination treatments using *G* tests of independence with Williams' correction, and one-way analyses of variance (Sokal and Rohlf, 1981). Hand-pollination of *Tellima* and *Tolmeia* yielded fruit set ranging from 0 to 89%. In *Tellima,* outcrossed flowers set a high percentage of fruits as did naturally pollinated flowers. Fruit set in manually outcrossed and naturally pollinated flowers was significantly higher than for manual selfing, pollinator exclusion, or control treatments. Fruit set in hand-selfed *Tellima* flowers was higher but not significantly different from either the emasculated controls or the pollinator exclusion treatment. Exclusion of pollinators from flowers reduced fruit set to a level equal to that of the control (16%), a significant reduction in fruit set when compared to naturally pollinated flowers. Surprisingly, hybrid crosses with *Tolmeia* as the pollen donor resulted in 58% fruit set, a significantly greater proportion of fruits than for manual selfing, pollinator exclusion, or the control. *Tolmeia* was completely self-incompatible, with 61% fruit set in outcrossed flowers, and no fruits set

TABLE 3. The effects of pollination treatment, plant, and flower position on seed set in *T. grandiflora* and *T. menziesii.* A multiway ANOVA compared mean seeds per fruit. Interactions between independent variables were not significant ($P > 0.05$) for *Tellima,* and were dropped from the model.

Source of variation	df	Mean square	*F*	*P*
Tellima				
Pollination	5	480761.404	32.390	0.0001
Plant	1	2099.470	0.141	0.7071
Flower position	1	22288.251	1.502	0.2214
Residual	294	14842.802		
Tolmeia				
Pollination	5	4974.741	8.103	0.0001
Plant	1	2711.376	4.416	0.0366
Flower position	1	431.714	0.703	0.4025
Pollination × plant	5	1034.824	1.686	0.1387
Pollination × flower	5	1499.378	2.442	0.0350
Pollination × plant × flower	6	2345.496	3.820	0.0012
Residual	243	613.931		

TABLE 4. The effects of six pollination treatments on fruit and seed set in *T. grandiflora* and *T. menziesii.* Seeds per fruit (excluding dropped flowers) and seeds per flower (including all pollinated flowers) are tabulated separately. A pairwise *G* test of independence compared fruit set among pollination treatments, using a simultaneous test procedure. *G* values are adjusted by Williams' correction. A one-way ANOVA with Scheffé's *F* test compared seed set among the pollination treatments. See Table 3 for results of the multiway ANOVA.

Pollination treatment	Fruit set			Seeds/fruit ($\bar{X} \pm$ SD)	Seeds/flower ($\bar{X} \pm$ SD)
	Fruits (*N*)	Flowers (*N*)	%		
Tellima					
Natural	55	66	83[a,b]	229 ± 131	191[a] ± 147
Outcross	41	46	89[a]	333 ± 116	296[a] ± 152
Self	12	47	25[c,d]	233 ± 153	59[b] ± 127
× *Tolmeia*	25	43	58[b,d]	135 ± 122	78[b] ± 114
Control	8	49	16[c]	231 ± 165	38[b] ± 107
Exclusion	8	51	16[c]	106 ± 74	17[b] ± 48
Tolmeia					
Natural	50	66	79[a]	60 ± 27	46[a] ± 34
Outcross	25	41	61[a]	91 ± 37	56[a] ± 53
Self	—	36	0[b]	—	—
× *Tellima*	3	35	9[b]	27 ± 30	2 ± 10
Control	1	36	3[b]	1	0
Exclusion	—	64	0[b]	—	—

[a–d] Matching letters indicate no significant difference ($P > 0.05$); with significance assessed separately for the two species.

in hand-selfed flowers. Hybrid crosses with *Tellima* as the pollen donor were only marginally successful and not significantly different from the controls, with three fruits resulting from 35 pollinations. For *Tolmeia* flowers, fruit set in naturally pollinated flowers was high and comparable to that for manually outcrossed flowers. Pollinator-excluded flowers did not set fruit.

Seed set within fruits also varied considerably by treatment, with a maximum of 559 seeds in an outcrossed *Tellima* fruit. Two measures of seed set are presented in Table 4, the first including only the mature fruits, and the second including all pollinations per treatment. Hand-outcrossed *Tellima* pollinations yielded the highest seed set, followed by naturally pollinated flowers, hybrid crosses, self-pollinated flowers, and control flowers. Excluding aborted flowers from the data changed slightly the rank of the treatments. Manually outcrossed seeds still had the greatest mean number of seeds per fruit. Self, control, and natural pollinations had higher levels of seed set than the hybrid pollinations, and the pollinator exclusion treatment had the lowest seed set. Outcrossed *Tolmeia* flowers produced slightly but not significantly more seeds per fruit on average than naturally pollinated flowers, and the difference was more dramatic with aborted flowers excluded. Manual selfing, control, and pollinator exclusion treatments produced no seed. *Tellima* × *Tolmeia* pollinations that produced fruit contained fewer seeds on average than outcrossed flowers.

The effect of increasing outcrossing distance on seed set was examined by ANOVA with regression for log outcrossing distance (1, 10, 100, and 1 000 m) vs. the number of seeds per fruit. The results of the regression were not significant for *Tellima* pollinations ($r^2 < 0.001$, $F < 0.001$). For *Tolmeia,* the number of seeds per fruit increased significantly as a function of outcrossing distance ($r^2 = 0.193$; $F = 7.155$; $P < 0.05$).

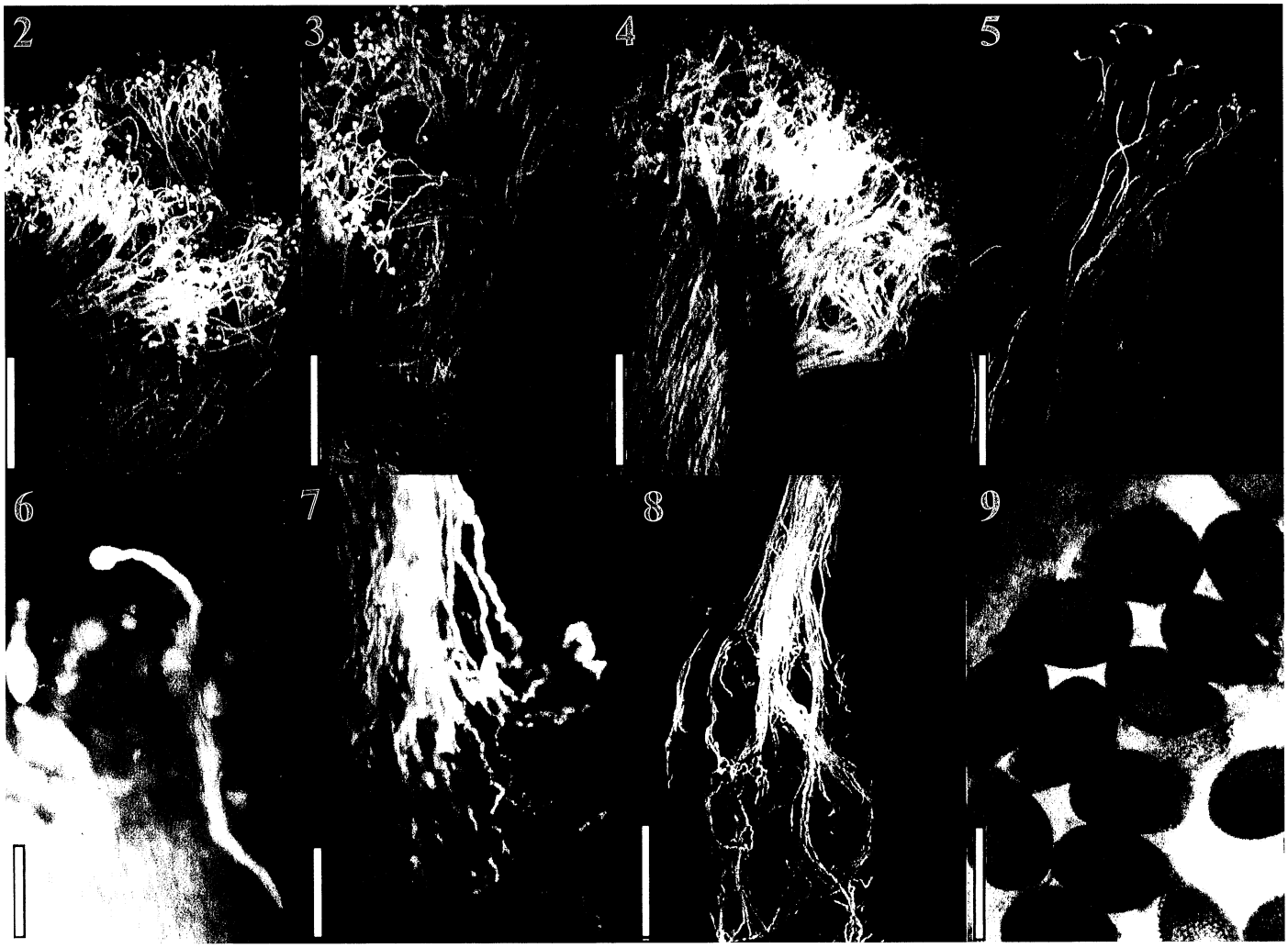
Pollen-style interactions—Self, outcross, and intergeneric pollen germinated at the stigmatic surface and grew through the upper style without inhibition in both species. Pollen tubes reached the lower style or ovary within 24 h after pollination. In *Tellima* and *Tolmeia,* manual pollinations including outcross, self, and *Tolmeia* produced more pollen tubes in the upper style than the control treatment. We measured no difference between the abundance of self and outcross pollen tubes (as estimated by a scoring procedure based on the logarithm of pollen tube number). In *Tolmeia,* no differences in the abundance of self, outcross, and *Tellima* pollen tubes were detected, even in the lower style (Weiblen, 1992). Photographs of pollen tubes under UV-fluorescence further illustrate pollen tube growth (Figs. 2–9). Figures 2–5 show pollen tubes in *Tolmeia* gynoecia for outcross, self, *Tellima,* and control pollinations, respectively. Germination and growth of an individual pollen grain are evident in Fig. 6. Figure 7 illustrates self-incompatibility in *Tolmeia* through the rejection of self pollen tubes at the lower style. Pollen tubes revealing parietal placentation and crassinucellate ovules after fertilization are shown in Figs. 8, 9.

Isozyme variation—The five isozymes examined by starch gel electrophoresis (LAP, MDH, PGI, PGM, and SkDH) yielded seven polymorphic loci. Allele frequencies at each locus are summarized for *Tolmeia, Tellima,* and synthetic and natural hybrids in Table 5. *Tellima* and *Tolmeia* shared alleles at LAP, MDH-1, MDH-3, PGM-2, and SkDH, but differences were detectable at PGI-1, PGI-2, and MDH-2. *Tellima* was monomorphic at all loci except SkDH, whereas polymorphic *Tolmeia* showed additional alleles not present in *Tellima.* For example, *Tellima* and *Tolmeia* shared PGM-2a, but this allele was less common in *Tolmeia* than the unique PGM-2b allele (Fig. 10). *Tellima* and *Tolmeia* were fixed for different allozymes at PGI-1, and were monomorphic for MDH-2a and MDH-2b, respectively. While *Tellima* was fixed for PGI-2c, *Tolmeia* had three unique PGI-2 alleles. A clone of the natural hybrid confirmed by Soltis and Bohm (1985) was also analyzed electrophoretically. The natural hybrid was heterozygous at each locus differentiating *Tolmeia* and *Tellima* and showed some additional alleles present in *Tolmeia* but not in *Tellima* (Fig. 10).

The progeny of hybrid crosses was grouped according to allozyme patterns that were either consistent with patterns predicted for *Tolmeia* × *Tellima* or were identical to *Tellima* (Table 5). Thirty-three of 40 putative "hybrids" were monomorphic at all enzyme loci except SkDH, and were allozymically identical to *Tellima,* the maternal parent. The seven remaining plants were heterozygous at the loci that distinguish *Tellima* and *Tolmeia* (PGI-1, PGI-2, and MDH-2). In addition to sharing the distinctive alleles of either species at allozymically differentiated loci, these plants as a group possessed alleles present in *Tolmeia* but not in *Tellima.* These confimed hybrids were often heterozygous at PGM-2, possessing PGM-2b, an allele not present in the maternal parent (Fig. 10). Some hybrid genotypes consisted of three SkDH alleles in a single plant, two from *Tolmeia* and one from *Tellima.*

Table 6 summarizes genetic diversity statistics including the mean number of alleles per polymorphic locus (A), the proportion of polymorphic loci (P), and the mean heterozygosity (H) for *Tellima, Tolmeia,* and the synthetic hybrids. Mean heterozygosity was calculated as the proportion of heterozygotes for each locus averaged over all loci. *Tolmeia* had more polymorphic loci, more alleles per locus, and higher heterozygosity than *Tellima.* Synthetic and natural hybrids were more polymorphic and heterozygous than either parent. Genetic identities based on the allozyme data for *Tellima, Tolmeia,* and the hybrids are compared in Table 7 (Nei, 1972). For the eight loci analyzed, *Tolmeia* and *Tellima* are distinct but retain some allozymic similarity. One group of putative "hybrids" was genetically identical to *Tellima.* The remaining synthetic hybrids were not different from the natural hybrid, which had isozymic affinities with both parents.

Hybrid morphology—The hybrids were also intermediate with respect to vegetative and floral morphology. Hybrids had basal cordate leaves similar to *Tellima* but also the vegetative plantlets at the base of the leaf blades, which are unique to *Tolmeia.* Flowers of the synthetic hybrids were nearly actinomorphic, with equal numbers of sepals, petals, and anthers (Fig. 1). Morphological features in the synthetic hybrids, such as the number, arrangement, shape, and color of the floral parts, showed intermediacy. For example, synthetic hybrids possessed



Figs. 2–9. Pollen germination and pollen tube growth in *Tolmeia* gynoecia, revealed by UV-fluorescent aniline blue stain. Pollen tubes in the stigma and style of (**2**) outcross, (**3**) self, (**4**) intergeneric, and (**5**) control pollinated flowers. **6.** A single pollen tube at the stigmatic surface. **7.** Self pollen is rejected in the lower style of self-incompatible *Tolmeia.* **8.** Outcross pollen tubes encircle the ovules, revealing parietal placentation in *Tolmiea.* **9.** Crassinucellate *Tolmeia* ovules after fertilization. In Figs. 1–5, 8–9 scale bars = 0.5 mm; in Figs. 6–7 scale bars = 0.1 mm.

five anthers per flower, compared to ten and three in *Tellima* and *Tolmeia,* respectively.

DISCUSSION

Mating systems—The results of hand-pollinations suggest a completely self-incompatible mating system in *Tolmeia,* and a mixed selfing-and-outcrossing mating system in *Tellima.* Pollinator observations and exclusion experiments suggest that outbreeding in *Tolmeia* is mediated by bumble bees, while mixed mating in *Tellima* is facilitated by small beetles at our Oregon field site (Table 2). Seed set from manual outcrossing compared to natural outcrossing suggests that pollinator activity may affect reproductive output in *Tolmeia,* as is sometimes the case in obligately outcrossed species (Bierzychudek, 1981). However, lower natural levels of seed set compared to hand-pollination could also result from near-neighbor mating and inbreeding depression, or the clogging of the stigma by self pollen. Patterns of resource availability and allocation can also impose limitations on seed set.

Evidence from floral morphology and phenology provide additional information on the mating system differences between *Tolmeia* and *Tellima.* The species differ with regard to the timing of anthesis and stigma receptivity, and the extent of stigmatic exsertion. Acropetalous floral development in both species insures that flowers in several stages of development are present on an inflorescence at any given time. *Tolmeia* is protandrous with anthesis occurring some days prior to the exsertion and receptivity of the stigma. Anthesis is also staggered between the inner and outer anthers, such that pollen is available in a single flower for at least two consecutive days. Pollen is offered to bees from only a few flowers per inflorescence per day, which promotes pollen transfer among plants by forcing bees to move among flower patches. Delayed exsertion and receptivity of the stigma reduce the opportunity for autogamous self-pollination. The opportunity for geitongamous selfing is reduced through a combination of bumble bee foraging behavior, protandry, and acropetaly. Bees at an inflorescence typi-

TABLE 5. Allele frequencies at eight enzyme loci in *T. grandiflora* and *T. menziesii.* Presumptive hybrids were grouped according to allozyme patterns either identical to *Tellima* or consistent with previously confirmed *Tolmeia* × *Tellima.* The number of individuals with interpretable banding patterns are reported for each locus (*N*). LAP and SkDH occasionally showed no activity or streaked patterns that were difficult to score. PGM-1 was too faint to be scored, whereas PGI-1, PGI-2, and PGM-2 were resolved consistently. *M* indicates missing data.

Gene	Allele	*Tolmeia*	*N*	Natural hybrid	Synthetic hybrid	*N*	"Hybrid" = *Tellima*	*N*	*Tellima*	*N*
LAP	a	0.17	5	0.50	0.67	3	1.00	5	1.00	4
	b	0.75		0.50	0.33					
	c	0.08								
MDH-1	a	1.00	23	1.00	1.00	7	1.00	19	1.00	24
MDH-2	a		23	0.50	0.50	6	1.00	17	1.00	26
	b	1.00		0.50	0.50					
MDH-3	a	0.16	16	0.50	0.50	6	1.00	12	1.00	16
	b	0.62		0.50	0.25					
	c	0.22			0.25					
PGI-1	a	1.00	17	0.50	0.50	7		12		19
	b			0.50	0.50		1.00		1.00	
PGI-2	a	0.12	17		0.40	5		10		17
	b	0.73		0.50	0.20					
	c			0.50	0.40		1.00		1.00	
	d	0.15								
PGM-2	a	0.21	34	0.50	0.57	7	1.00	33	1.00	40
	b	0.79		0.50	0.43					
SkDH	a		19	M	0.17	3	0.36	22	0.26	25
	b	0.63			0.33					
	c	0.12			0.42		0.64		0.74	
	d	0.20			0.08					
	e	0.05								

cally visit the basal flowers first and then move toward the apical flowers. This increases the opportunity for deposition of outcross pollen on receptive stigmas followed by removal of pollen from the same inflorescence.

In contrast to *Tolmeia,* anthesis and stigma receptivity are simultaneous with the opening of the *Tellima* flower. Furthermore, the anthers and receptive stigmas are in close physical proximity within the flower. Stylar exsertion does not occur, and the receptive stigmatic surfaces, located on the sides of the capitate stigma, are perfectly level with the anthers. Anthesis is not staggered and the longevity of the pollen grains appears to be short. Since few flowers per inflorescence contain fresh pollen at any given time, pollen-collecting insects are required to forage among inflorescences. These observations would seem to support a mixed mating system, where within-flower self-pollination is supplemented by occasional outcrossing. Miller (1985) described gynodioecious *Tellima* populations from the southern part of its range, but we did not observe male sterility in *Tellima* at the Sandy River Gorge.

Fig. 10. Photograph of a starch gel showing PGM-2 banding patterns for *T. menziesii, T. grandiflora,* and their hybrid. *Tolmeia* (lanes 1 and 2) is heterozygous at PGM-2, sharing alleles 2a and 2b. *Tellima* (lanes 7 and 8) is homozygous for PGM-2a. A confirmed, natural hybrid is heterozygous (lanes 5 and 6). Two putative hybrids in lanes 3 and 4 have different banding patterns. Lane 3 is heterozygous, containing the PGM-2b allele not present in maternal *Tellima.* Lane 4 is homozygous for PGM-2a, consistent with pure *Tellima.*

Differences in the mating systems of *Tolmeia* and *Tellima* are also reflected in their pollen: ovule ratios. The ratio of pollen grains to ovules is a coarse measure of how much reproductive resource is allocated to male and female functions (Cruden, 1977). The amount of resource allocated to male reproductive function may reflect the efficiency of pollen transport and the degree of outcrossing in the mating system. Primarily outcrossing species generally have higher pollen : ovule ratios than selfing populations. The *Tolmeia* pollen : ovule ratio is nearly double that of *Tellima* (Table 1), suggesting that *Tolmeia* is more highly outbreeding than *Tellima.* Another correlate of plant mating systems is the level of genetic variation within populations as estimated by enzyme electro-

TABLE 6. Mean number of alleles per polymorphic locus (*A*), proportion of polymorphic loci (*P*), and mean heterozygosity (*H*) for *Tolmeia menziesii* and *Tellima grandiflora.* Presumptive hybrids were grouped according to allozyme patterns either consistent with previously confirmed *Tolmeia* × *Tellima* or identical to *Tellima.*

Genetic diversity	*A*	*P*	*H*
Tellima	2.0	0.125	0.045
"Hybrid" = *Tellima*	2.0	0.125	0.023
Synthetic hybrid	2.6	0.875	0.875
Natural hybrid	2.0	0.875	0.857
Tolmeia	3.0	0.625	0.317

TABLE 7. Mean genetic identities (*I*) of *Tellima, Tolmeia,* and hybrids.

Genetic identity (*I*)	*Tellima*	"Hybrid" = *Tellima*	Synthetic hybrid	Natural hybrid
"Hybrid" = *Tellima*	1.00			
Synthetic hybrid	0.73	0.84		
Natural hybrid	0.74	0.78	0.97	
Tolmeia	0.24	0.24	0.84	0.73

phoresis (Hamrick and Godt, 1990). The proportion of polymorphic loci (*P*) and mean heterozygosity (*H*) for a species typically varies according to the frequency of different types of mating. High levels of variation are generally associated with primarily outcrossed species and low levels with selfed species. *P* and *H* are substantially higher for *Tolmeia* than for *Tellima.* Rieseberg and Soltis (1987) suggest that low variation in *Tellima* may be due to selfing as the primary mode of reproduction. Our results suggest that monomorphic *Tellima* is characterized by at least mixed outcrossing and selfing, while polymorphic and heterozygous *Tolmeia* is obligately outcrossing.

Pollen–style interactions and self-incompatibility—Our observations suggest that self-incompatibility in *Tolmeia* is late-acting, consistent with the observations of Sears (1937). Late-acting self-incompatibility has been noted as characteristic of hollow-styled species because pollen tubes proceed down the style without intimately interacting with the stylar tissue (Seavey and Bawa, 1986). This appears to be the case in *Tellima* and *Tolmeia,* as has also been noted in other hollow-styled relatives of the Saxifragaceae (Arasu, 1970). Pollen tube attrition was noted in the lower style, as indicated by disoriented pollen tubes (Fig. 7), but some self pollen tubes escaped rejection and proceeded to the ovary, occassionally reaching the micropyle (Fig. 8).

Confirmation of hybridization—Hand-pollinations did not eliminate agamospermy or contamination by intraspecific pollen as alternative sources of parentage of the putative hybrids. Pollen tube growth and seed set in the emasculated *Tellima* controls suggest that some contamination occurred. However, *Tolmeia* × *Tellima* pollinations resulted in significantly higher fruit set than did *Tellima* controls, despite comparable mean numbers of seeds per fruit between these treatments (Table 3). Germination of the progeny resulting from hybrid pollinations was low (33%) compared to controls (78%), suggestive of hybrid seed inviability. Furthermore, isozymes unambiguously demonstrated that the collection of putative hybrids propagated from seed was highly impure (Table 5). Thirty-three of 40 progeny from hybrid crosses were allozymically identical to the maternal parent, which we attribute to intraspecific pollen contamination or agamospermy. The remaining seven plants were indeed hybrids, as confirmed by their heterozygosity at enzyme loci differentiating *Tolmeia* and *Tellima.* Vegetative and floral morphological evidence provide additional support for hybridity (Fig. 1). Hybrid pollen shows reduced germination on artificial media compared to *Tolmeia* and *Tellima* (K. Karoly, letter of 6 May 1994). We believe that the synthetic hybrids from northern Oregon and their natural counterparts are likely to be sterile due to irregular chromosome numbers ($2n = 21$). Hybrids are expected to differ in chromosome number from the parental species since *Tellima* is $2n = 14$ and tetraploid *Tolmeia* is $2n = 28$. Consistent with our triploid expectation, some synthetic hybrids shared three alleles at the SkDH locus. Two naturally occurring hybrids, one from southern Oregon ($2n = 14$) and the other from southern Washington ($2n = 21$), were consistent with the distribution of diploid and tetraploid *Tolmeia* populations. Soltis, Soltis, and Ness (1990) produced triploid *Tolmeia* by interbreeding diploid and tetraploid plants, however, allopolyploidy has not been observed.

Reproductive isolating mechanisms—Successful hybridization by experiment begs an explanation for the rarity of natural *Tolmeia* × *Tellima.* Evidence from pollinator observations, hand-pollinations, pollen tube growth, and hybrid performance suggest that several isolating mechanisms are important. Closely related perennial herbs can be reproductively isolated by ecological, phenological, or ethological factors (Grant, 1981). In addition to these premating isolating mechanisms, postmating mechanisms can include barriers to crossability, pollen–pistil incompatibility, and hybrid inviability and sterility (Stebbins, 1966). Our results point to a series of ethological, stylar, and postzygotic barriers to hybridization. Geography and flowering phenology are not likely barriers, since the species overlap in these respects. The species commonly occupy microsites that differ in soil moisture, although they sometimes occur as nearest neighbors. Morphological differences could serve as ethological or mechanical barriers to hybridization by discouraging the movement of pollinators between species. The flowers of *Tellima* and *Tolmeia* (Fig. 1) and their insect visitors (Table 2) are suggestive of different pollination mechanisms. However, we did not observe complete pollinator specificity at our study site. *Bombus edwardsii,* the principal pollinator of *Tolmeia,* was observed visiting *Tellima* on two occasions, providing the opportunity for occasional transfer of pollen between species. Reproductive isolation probably does not occur at any single level but through the effects of subtle microhabitat and ethological divergence, combined with the limited success of hybrid fruit set, seed set, hybrid inviability, and sterility. Natural hybrids, however, can be maintained clonally through vegetative propagation.

In summary, evidence from floral morphology and phenology, pollinator interactions, and self-incompatibility suggest that sexual reproduction in *Tolmeia* consists entirely of outcrossing mediated by bumble bees. The mating system of *Tellima* is mixed, where the arrangement of floral parts and presence of rove beetles in the flowers insures the opportunity for selfing occasionally supplemented by outcrossing. Morphological divergence and pollinator specificity do indeed contribute to the reproductive isolation of sympatric *Tolmeia* and *Tellima.* The radiation of the Saxifragaceae into many species with different distributions, habitat requirements, and floral morphologies presumably involved the reproductive isolation of progenitor populations. Recent molecular phylogenies of the *Heuchera* group (Saxifragaceae sensu stricto) suggest that *Tolmeia* and *Tellima* are not sister

taxa, and substantial molecular, chemical, and morphological change has occurred since their respective lineages shared a common ancestor (Soltis et al., 1991b). Soltis et al. (1993) suggest that reductions and modifications of the number of floral parts have occurred several times within the *Heuchera* group. The study of patterns of character evolution in the Saxifragaceae using phylogenies should provide additional insights into mating system evolution in this group. It might be possible, for example, to infer whether some floral traits evolved before or after the divergence of *Tolmeia* and *Tellima* lineages. In any case, it seems plausible that floral divergence and reproductive isolation reflect the evolution of different sexual strategies in the forms of pollinator relations and mating systems.

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