Heritability of Ornamental Foliage Characteristics in Diploid, Triploid, and Tetraploid *Hypericum androsaemum* L.

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Significance to Industry: Increasing awareness and concern about invasive plants (3) provides an opportunity for plant breeders to address this problem by developing seedless cultivars with improved ornamental characteristics. Studies investigating the heritability of variegated and purple leaf foliage types in *H. androsaemum* indicated that both are simple recessive traits at the diploid level and are not linked. This has led to the recovery of a novel phenotype combining the variegated foliage of ‘Glacier’ with the purple foliage of ‘Albury Purple’. This novel phenotype was also recovered at the triploid and tetraploid level. The lack of a triploid block in *H. androsaemum* allowed the recovery of triploid progeny that are currently being evaluated for fertility and ornamental traits. Triploids are typically highly infertile (4) and the development of these plants will facilitate the release of a series of seedless *H. androsaemum* with a range of ornamental foliage types. The present research demonstrates the feasibility of breeding simultaneously for ornamental traits and non-invasiveness.

Nature of Work: Hypericums, or St. Johnsworts, are popular summer flowering, deciduous or semi-evergreen, shrubs and perennials. The genus *Hypericum* [Clusiaceae (Guttiferae)] contains approximately 425 species distributed worldwide (1, 2). Of the species represented in the nursery trade, *H. androsaemum* (tutsan St. Johnswort) and its hybrids are notable for their floriferous and hardy nature and cultivars with unique foliage and fruiting characteristics. *Hypericum androsaemum* is native from Western Europe, through the Mediterranean, and into Northern Iran. Unfortunately, it is now also found naturalized in Australia, New Zealand, and Chile (2) and is a potential problem in the U.S. Pacific Northwest (Dan Hinkley, pers. comm.). This semi-evergreen shrub grows less than three feet in height and has abundant, yellow, star-like flowers and fleshy red fruit which mature to a brown capsule. *Hypericum androsaemum* performs best as a landscape shrub in the cooler parts of U.S.D.A. zones 6-8. Within the genus *Hypericum* it is the only species that contains both variegated and purple foliaged cultivars: *H. androsaemum* ‘Glacier’ with highly variable, white-mottled leaves and ‘Albury Purple’ with wine-purple foliage and red-veined yellow flowers. In order to initiate a breeding program for the simultaneous development of ornamental and non-invasive Hypericum, it is desirable to establish the mode of inheritance for these foliage traits, of which little is known for woody ornamental plants. The objective of this study was to investigate the mode of inheritance for two different foliage types (variegated and purple) at three different ploidy levels (diploid, triploid, and tetraploid) in *Hypericum androsaemum*.

Plants of *H. androsaemum* 'Glacier' (G), 'Albury Purple' (AP), and hybrids were grown and maintained in greenhouses at the Mountain Horticultural Crops Research Station, Fletcher, N.C. Segregation ratios were determined for diploid crosses in reciprocal di-hybrid F_1 and F_2 families and backcrosses to each parent (see Table 1). Tetraploid segregation ratios were calculated for $F_{2[4x]}$ from selfed autotetraploid G × AP F_1's ($F_{1,1[4x]}$). Triploid segregation ratios were determined from crosses between autotetraploid G × AP ($F_{1,1[4x]}$) and diploid G × AP ($F_{1,1[2x]}$). For all crosses, flowers were emasculated prior to anthesis. Pollen was collected from recently dehisced anthers and either used fresh or dried overnight at 41°F (5°C) using indicator drierite (Drierite, Xenia, Ohio), and stored at 41°F (5°C) for use in subsequent crosses. Pollen was applied to stigmas daily using small brushes until stigmas turned brown after ≈ 10 days. Fruit were collected when capsules turned from bright red to brown and were dried at room temperature for 1-3 days. Seeds were separated and sown onto the surface of a 1 peat : 1 vermiculite (by volume) medium and misted regularly until germination occurred in 1 to 4 weeks. Seedlings were transplanted into 40-cell trays containing seedling medium and remained there until phenotype scoring. Phenotypes were scored when seedlings had > 3 sets of true leaves. Heritability of variegated and purple leaf traits was tested on the hypothesis that both traits were inherited in a simple Mendelian recessive manner. Chi-square analysis was conducted on segregating families ($F_{2,1[2x]}$, $F_{2,2[2x]}$, $BC_{1,1}$, $BC_{1,2}$, $F_{2[3x]}$, and $F_{2[4x]}$). The Chi-square test of independence for linkage was calculated for diploid F_2's ($F_{2,1[2x]}$ and $F_{2,2[2x]}$). All crosses were conducted during the summers of 2003 and 2004.

Results and Discussion: Reciprocal di-hybrid crosses were performed to rule out maternal affects on inheritance. In both G × AP and AP × G F_1's, all seedlings were green (data not shown), demonstrating that neither traits were maternally inherited via extra-nuclear plastids. Furthermore, the lack of variegation and purple pigment in both F_1 crosses rules out incomplete dominance for each trait. Segregation ratios for the diploid F_2's approximated the predicted ratio of phenotypic classes of 9 wild type (green) : 3 variegated : 3 purple : 1 variegated and purple; supporting our hypothesis of simple recessive inheritance for both traits (Table 2). The chi-square tests of independence for linkage (χ^2=1.75, P = 0.19 and χ^2=0.02, P = 0.89 for G × AP and AP × G, respectively) reveal no evidence for linkage between the two traits.

Backcross data generally supports the simple recessive model for both traits, though for $BC_{1,2}$ we had a few spontaneous variegated seedlings occur when we expected a 1 green : 1 purple in the progeny (Table 2). The presence of the variegated types is not explained by accidental selfing or pollen contamination. At higher ploidy levels, expression of simple recessive traits becomes less frequent due to a lower probability of homozygosity with increased numbers of alleles from duplicate chromosomes. At the triploid level, the data was an excellent fit to the simple recessive model (χ^2=1.38, P = 0.71) (Table 2). However, at the tetraploid level, we observed a greater number of variegated phenotypes than predicted, and thus had a poor fit to our model (χ^2=141.2, P < 0.001) (Table 2). This lack of fit was not explained by random chromosome or chromatid assortment at the variegated locus (data not shown) and is difficult to explain in light of the lack of deviation at the purple locus. The greater than expected expression of variegated phenotypes at the tetraploid level may indicated a partial shift from qualitative to quantitative inheritance or other modifying genetic factors. This research is continuing to evaluate fertility of these hybrids.

Literature Cited:

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Table 1. Crosses between *Hypericum androsaemum* 'Glacier' (G) and 'Albury Purple' (AP) and families produced.

Cross	Ploidy	Families
G × AP	2x	$F_{1,1[2x]}$, $F_{2,1[2x]}$
AP × G	2x	$F_{1,2[2x]}$, $F_{2,2[2x]}$
G × $F_{1,1[2x]}$	2x	$BC_{1,1}$
AP x $F_{1,1[2x]}$	2x	$BC_{1,2}$
$F_{1,1[4x]}$ × $F_{1,1[2x]}$	3x	$F_{2[3x]}$
$F_{1,1[4x]}$ × self	4x	$F2_{[4x]}$

Table 2. Segregation for *Hypericum androsaemum* foliage color types in families derived from 'Glacier' (G) and 'Albury Purple' (AP).

		Progeny (no. seedlings)[z]				Expected		
Cross	Family	Green	Var.	Purple	V/P	Ratio	x^2	*P*
G × AP	$F_{2,1[2x]}$	218	71	88	20	9:3:3:1	3.69	0.30
AP × G	$F_{2,2[2x]}$	214	77	81	28	9:3:3:1	1.43	0.70
G × $F_{1,1[2x]}$	$BC_{1,1}$	200	196	--	--	1:1	0.04	0.84
AP x $F_{1,1[2x]}$	$BC_{1,2}$	178	4[y]	221	3[y]	1:1	4.63[y]	0.03
$F_{1,1[4x]}$ × $F_{1,1[2x]}$	$F_{2[3x]}$	573	46	49	3	121:11:11:1	1.38	0.71
$F_{1,1[4x]}$ × self	$F_{2[4x]}$	701	76	22	1	1225:35:35:1	141.2	<0.001*

[z]Number of progeny for each phenotypic class (Green = wild type; Var. = variegated type; Purple = purple type; V/P = variegated and purple combination).

[y]Unexpected variegation, which can not be explained by accidental self- or cross-pollination. These seedlings were not used to calculate the chi-square statistic (x^2).