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Inheritance of Flowering Habit in Russian Dandelion

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Abstract. **Russian dandelion [*Taraxacum kok-saghyz* (TKS)] is a latex-producing, temperate species that has the potential to be grown as a source of natural rubber in North America. Flowering habit varies within the species; winter-type plants require a cold period or vernalization to flower, whereas spring-type plants flower without this treatment. Because flowering habit is correlated with rubber yield, understanding the genetic factors governing the trait would be useful for breeding. The objective of this research was to determine the inheritance of vernalization requirement in TKS. Winter-type and spring-type plants were intercrossed to create the F_1, F_2, and backcross generations and progeny segregation ratios were analyzed. A genetic model with three major genes is proposed, where a dominant allele at locus *A*, in combination with homozygous recessive alleles at either or both of two loci, *B* and *C*, confers winter type, whereas spring type is conferred by homozygous recessive alleles at *A*, regardless of genotype at *B* or *C*, or dominant alleles at *A*, *B*, and *C*.**

Russian dandelion [*Taraxacum kok-saghyz* (TKS)] is an herbaceous perennial that can also be grown as an annual. Latex can be found in the root, in specialized cells called laticifers (Whaley and Bowen, 1947) and this species could be a source of natural rubber, essential for the fabrication of over 40,000 products vital to industries including transportation, health care, and construction (Mooibroek and Cornish, 2000). TKS grows well in southern Ontario and the northern United States, and it is currently under development as a new crop to introduce natural rubber production to these regions (van Beilen and Poirier, 2007).

The transition from vegetative to reproductive development in plants is cued by endogenous, as well as environmental signals, such as photoperiod and temperature (Thomas et al., 2006). TKS can require a period of cool temperatures, known as vernalization, to induce flowering (Borthwick et al., 1943). This is common in temperate perennials because it encourages flowering after winter, during the favorable conditions of spring (Andres and Coupland, 2012). Although the natural distribution of TKS is restricted to a relatively small area along the Alatau mountain range in Kazakhstan, the species can grow in a number of regions with favorable climates (Whaley and Bowen, 1947).

Variation for flowering habit is observed in natural populations. Early flowering, spring-type, plants do not require vernalization and flower ≈50 d after planting in a greenhouse with a 16-h photoperiod (K.J.M. Hodgson-Kratky and D.J. Wolyn, unpublished data). Winter-type plants, in contrast, grow vegetatively and generally will not flower without a cold period (Hodgson-Kratky et al., 2015).

The floral induction pathway has been studied extensively in the model plant, *Arabidopsis thaliana*. In this species, two major loci determine flowering habit: *flowering locus C* (*FLC*) and *frigida* (*FRI*) (Koornneef et al., 1994). Winter-type plants carry dominant functional alleles at each of these two loci, and homozygous recessive genotypes with inactive *fri* and/or *flc* alleles result in early flowering (Gendall and Simpson, 2006; Johanson et al., 2000). Control of flowering through *FLC*/*FRI* is conserved in many plant species (Irwin et al., 2012; Kuittinen et al., 2008; Reeves et al., 2007; Risk et al., 2010; Schranz et al., 2002; Zhang et al., 2009).

The genetic pathway controlling the vernalization requirement for flowering in temperate cereals, such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and rye (*Secale cereale*), evolved independently of that in *A. thaliana*, and is regulated primarily by three genes: *Vernalization 1* (*VRN1*) (Yan et al., 2003), *VRN2* (Yan et al., 2004), and *VRN3* (Yan et al., 2006). Spring-type plants possess recessive null mutations at the *VRN2* locus (Dubcovsky et al., 2005; Yan et al., 2004) or dominant mutations in the promoter region of *VRN1* (Dubcovsky et al., 2005; Fu et al., 2005; Tranquilli and Dubcovsky, 2000; Yan et al., 2004) or *VRN3*, which cause high expression in these two genes regardless of environmental conditions (Yan et al., 2006).

Flowering habit is an important trait in TKS breeding because winter-type plants have higher rubber yields than spring-type plants (Whaley and Bowen, 1947). Therefore, populations under development for high rubber are also selected for vernalization requirement and thus it would be useful to understand the inheritance of flowering habit. Based on the genetic pathways identified in *A. thaliana* and cereals, multiple interacting loci influencing the trait are predicted in TKS. The objectives of this research were to determine the number of major genes controlling the vernalization requirement in TKS and interlocus interactions.

Materials and Methods

Genetic materials and analysis. TKS accessions (W6 35156, W6 35159, W6 35160, W6 35162, W6 35164, W6 35165, W6 35166, W6 35168, W6 35169, W6 35170, W6 35172, W6 35173, W6 35176, W6 35177, W6 35178, W6 35179, W6 35180, W6 35181, W6 35182, W6 35183) were obtained

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from the Washington State University Regional Plant Introduction Station of the Agricultural Research Service (Pullman, WA), a division of the U.S. Department of Agriculture, and two populations were developed: one with high rubber content and the second with rapid flowering. To create the high rubber population that segregated for vernalization requirement, 100 seeds from each accession were planted and 100 plants with the highest rubber content were intercrossed. The rapid-flowering population was developed by randomly mating spring-type plants observed in the TKS accessions and conducting three cycles of phenotypic recurrent selection for early flowering.

Five winter-type (W) plants, requiring vernalization, were selected randomly from the high-rubber population to reciprocally cross with five spring-type (S) plants, not requiring vernalization, from the rapid-flowering population to create the first generation for analysis. Reciprocal crosses were also performed within each group of plants (W and S). For all crosses, progeny from each parent were observed every 1–2 d and flowering time was recorded when all florets on the first flower head were open. Plants were observed for 20 weeks and two phenotypes were detected. Spring-type plants flowered before 90 d of growth; winter types generally did not flower without vernalization, but a small number flowered after 90 d without treatment. Half of the winter-type plants from each cross were placed at 4 °C for 6 weeks and then observed for flowering in the greenhouse to confirm vernalization response. Each of these plants flowered within 30 d of removal from the cold. The remaining plants were grown in the greenhouse for further observation. About 15% of these plants flowered at times ranging from 90–180 d of growth, proving that flowering could be induced by vernalization. Crosses were performed between and within the W and S phenotypic classes and full-sib families to produce the F_2. Backcrosses were also performed between the original parents and spring- and winter-type F_1 progeny.

Growth and culture. For each generation of genetic analysis, seeds were germinated in petri dishes with moistened filter paper. F_1 and F_2 seedlings were transplanted into 50-cell plug trays on 8 Oct. 2013 and 9 Apr. 2014, respectively, and then repotted 30 d later into 12.7-cm-diameter pots filled with peat-based medium (Sunshine LC1; Sun Gro Horticulture, Vancouver, BC, Canada). All plants were grown in a greenhouse with a 16-h photoperiod, using natural light supplemented with a photosynthetic photon flux density of 50–70 $\mu mol \cdot m^{-2} \cdot s^{-1}$ produced by high-pressure sodium lamps. Air temperature was maintained at 21/18 °C (day/night) during evaluation of the F_1, but rose to 24/20 °C during the F_2 evaluation due to high ambient temperature. Plants were fertilized on alternate weeks with 20N–3.5P–16.6K plus micronutrients (All Purpose High N fertilizer; Plant Products, Leamington, ON, Canada) at a concentration of $1.5\ g \cdot L^{-1}$.

Crossing methods. Open-ended 33.0 × 40.7-cm polypropylene microperforated bags (Elkay Plastic Co., Los Angeles, CA) were used to isolate plants individually. Flower heads (capitula) were rubbed together to transfer pollen and make a cross. Emasculation was unnecessary because plants are self-incompatible; however, unpollinated capitula of bagged plants were observed as controls for self-pollination and transfer of pollen by insects.

Statistical analyses. Phenotypic ratios of F_1 progeny from each of the two parents for each reciprocal cross were determined to be homogeneous or heterogeneous with chi-square heterogeneity tests (Bowley, 2008). Two of 17 reciprocal crosses were heterogeneous with phenotypic ratios of 1:3 (S:W) and 3:1, and 7:1 and 1:1 (data not shown), so they were not pursued further. All other F_1 as well as F_2 and backcross (BC_1) progeny reciprocal crosses were determined homogeneous so the phenotypic data from the two parents in each cross were pooled. Observed and expected segregation ratios were compared using the Pearson goodness of fit test, estimated with the PROC FREQ procedure in SAS (version 9.3; SAS Institute, Cary, NC). Since there were two phenotypic classes, the chi-square analysis was adjusted with Yate's correction for continuity (Bowley, 2008). Significance was determined at $P \leq 0.05$.

Results

Crosses between spring and winter phenotypes produced mostly spring-type F_1 progeny [Table 1 (crosses 1–5)], suggesting dominance for early flowering. Among crosses 1–5, 783 spring-type and 20 winter-type plants were observed. Intercrossing the spring-type parents produced only spring-type progeny (cross 6). If only one major gene determined the vernalization requirement, where spring type (*AA* or *Aa*) is dominant to winter type (*aa*), then only winter type (*aa*) would be expected in winter-type (*aa*) × winter-type (*aa*) crosses; however, this was not observed (crosses 7–9), suggesting the genetic control of flowering type involves more than one gene.

Several models were tested to understand the segregation patterns observed among three generations of crosses and one could explain all the data (Table 2). Spring- and winter-type flowering may be controlled by three interacting loci *A*, *B*, and *C*, where a dominant allele at locus *A* in combination with a homozygous recessive genotype at either or both of two loci, *B* and *C*, determines winter-type (e.g., *A-bbC-*, *A-B-cc*, and *A-bbcc*) and spring-type plants lack a dominant allele at *A* (e.g., *aa----*) or have a dominant allele at all three loci, *A*, *B*, and *C* (e.g., *A-B-C-*). To test this model, genotypes were proposed for each of the plants used in crosses based on progeny segregation observed throughout each generation.

Winter-type plants, W-1 and W-6 [Table 1 (cross 7)] were assigned the genotypes, *AabbCc* and *AaBbcc*, respectively, to account for the progeny segregating 7 spring type (*A-B-C-* and *aa----*):9 winter type (*A-bb--* and *A---cc*). Crosses among progeny from cross 7 were not successful likely due to shared self-incompatibility alleles. However, backcrosses performed between progeny and parents provided evidence for the proposed parental genotypes [Table 3 (crosses 10–12)]. Progeny from backcrossing two spring-type plants from cross 7 to parent W-1 (*AabbCc*) segregated 11 spring type (*AaBbC-* and *aa----*):5 winter type (*Aabb--* and *AaBbcc*) (crosses 10 and 11), suggesting the two spring-type plants used as parents were the *aaBbCc* genotype. Backcrossing a winter-type plant, 7–24, to W-6 (*AaBbcc*) resulted in 1:3 segregation (cross 12); 7–24 was assigned the genotype *AaBbcc*, whereby progeny segregated 1 *aa--cc*:3 *A---cc*.

Crossing the winter-type W-1 plant with the spring-type S-1 plant resulted in 156 spring-type and one winter-type progeny; the one winter-type plant was assumed to result from minor background genes segregating, and a ratio of 1:0 was used for the proposed three-gene model [Table 1 (cross 1)]. On the basis of the previous crosses, W-1 was assigned the genotype *AabbCc*, therefore, S-1 could be *AABBCC*, *AaBBCC*, or *aaBBCC* to produce only spring-type progeny. Intercrossing spring-type plants from W-1 × S-1 produced all spring-type progeny, as well [Table 3 (crosses 13 and 14)], which eliminates *AABBCC* as an option

Table 1. Genetic analysis of F_1 progeny from crosses among spring-type (S) and winter-type (W) russian dandelion plants.

Suggested model[z]	Cross no.	Cross[x]			Segregation ratio (S:W) Observed[y]	Segregation ratio (S:W) Expected	χ^{2w}	*P* value
aaBBCC or *AaBBCC* × *AabbCc* =*A-BbC-*, *aaBbC-*	1	S-1	×	W-1	156:1	1:0		
aaBBCC × *AaBBcc* =*AaBBCc*, *aaBBCc*	2	S-2	×	W-2	206:5	1:0	—	—
aaBBCC or *AABBCC* × *AAbbCc* =*AaBbC-* or *AABbC-*	3	S-3	×	W-3	93:4	1:0	—	—
aaBBCc × *AabbCC* =*AaBbC-*, *aaBbC-*	4	S-4[v]	×	W-4	144:0	1:0	—	—
aaBBCC × *AaBBcc* =*AaBBCc*, *aaBBCc*	5	S-5[u]	×	W-5	184:10	1:0	—	—
aaBBC- × *aaBBCC* =*aaBBCc*, *aaBBCC*	6[t]	S	×	S	331:0	1:0	—	—
AabbCc × *AaBbcc* =7*A-B-C-*, *aa----*:9*A-bb--*, *A---cc*	7	W-1	×	W-6	34:47	7:9 /1:1[s]	0.04 /1.78	0.83 /0.18
AAbbCc × *AaBBcc* =1 *A-BbCc*:1 *A-Bbcc*	8	W-3	×	W-2	65:58	1:1	0.29	0.59
AabbCC × *AaBBcc* =*AABbCc*, *AaBbCc*, *aaBbCc*	9	W-4	×	W-5	94:7	1:0	—	—

[z]Genotypes proposed based on observed segregation ratios in F_1, F_2, and backcross progeny; winter-type plants have genotypes: *A-bbC-*, *A-B-cc*, and *A-bbcc* and spring-type plants have genotypes: *A-B-C-* and *aa----*.

[y]For all crosses, seed was collected from both parents and analyzed as two separate populations that were then pooled together after they were determined homogeneous by chi-square heterogeneity tests. Pooled results are shown.

[x]Observed segregation ratios were compared with expected ratios using the chi-square statistic adjusted with Yate's correction for continuity.

[w]Letter preceding hyphen corresponds to phenotype; S for spring type and W for winter type. Number following hyphen is the individual plant identifier.

[v]S-4 can have the following genotypes: *aaBBcc*, *aaBBCc*, *aaBBCC*, *AaBBCc*, *AaBBCC*, *AABBCc*, and *AABBCC*.

[u]S-5 can have the following genotypes: *aaBBCC*, *aaBbCC*, *AaBBCC*, *AaBbCC*, *AABBCC*, and *AABbCC*.

[t]S-1 to S-5 were intercrossed; all four crosses were pooled together.

[s]Cross 7 also fits 1:1 ratio which can be explained by a two gene model where a homozygous recessive genotype at either of two duplicate loci *B* and *C* conferred the vernalization requirement; *bbCC* (W-1) × *Bbcc* (W-6) = 1 spring type (*BbCc*):1 winter type (*bbCc*).

Table 2. Flowering habit in russian dandelion is determined by the interaction of three genes *A*, *B*, and *C*, where a dominant *A* allele in combination with a homozygous recessive genotype at *B*, *C*, or both *B* and *C* confers winter type (W) and recessive alleles at *A*, regardless of genotypes at *B* or *C*, or dominant alleles at *A*, *B* and *C* confer spring type (S).

	BB	*Bb*	*bb*	
AA	S	S	W	*CC*
	S	S	W	*Cc*
	W	W	W	*cc*
Aa	S	S	W	*CC*
	S	S	W	*Cc*
	W	W	W	*cc*
aa	S	S	S	*CC*
	S	S	S	*Cc*
	S	S	S	*cc*

for S-1 because *AabbCc* (W-1) × *AABBCC* produces plants carrying a dominant allele at *A* and heterozygous at *B*, *A-BbC-*; intercrossing these (*A-BbC-* × *A-BbC-*) would result in one-quarter of the progeny with the late-flowering phenotype, *A-bb--*, which was not observed. For the remaining two genotypes possible for S-1, *AaBBCC*, and *aaBBCC*, all spring-type progeny with genotypes *A-BbC-* and *aaBbC-* would be produced when S-1 is crossed with W-1 (*AabbCc*) [Table 1 (cross 1)]. Crossing two *aaBbC-* progeny from cross 1 would explain the recovery of only spring-type progeny in crosses 13 and 14 (Table 3). Therefore, either genotype, *aaBBCC* or *AaBBCC*, could be assigned to S-1 [Table 1 (cross 1)]. Another cross between progeny from cross 1 [Table 3 (cross 15)] had a small sample size, so either a 1:0 or 7:1 ratio could fit the data. For a 7:1 ratio, the two parents would have genotypes *AaBbCC* and *aaBbCC*, producing seven spring-type plants with genotypes *A-B-CC* and *aa--CC*, and one winter-type, *AabbCC*, plant. To produce all spring-type progeny (*aa--CC*), the parents would both have the *aaBbCC* genotype (as shown in crosses 13 and 14).

Crosses between two winter-type plants and among their progeny validated the proposed model. Progeny segregated 1:1 when two winter types, W-3 and W-2, were intercrossed [Table 1 (cross 8)]. The genotypes *AAbbCc* and *AaBBcc* were assigned to W-3 and W-2, respectively, which would produce 1 spring type (*AABbCc* and *AaBbCc*):1 winter type (*AABbcc* and *AaBbcc*). When backcrossing a spring-type progeny (plant 8-19) to the W-2 parent, offspring segregated 5 spring type:3 winter type [Table 3 (cross 16)], which could result from a *AaBbCc* × *AaBBcc* (W-2) cross. Therefore, 8-19 was assigned the genotype *AaBbCc*.

Intercrossing progeny from cross 8 was not successful due to shared self-incompatibility alleles; therefore, crosses were made among the progeny from crosses 7 and 8 (Table 1). Crosses 7 and 8 produced offspring with 12 and 4 possible genotypes, respectively, and specific genotypes were assigned to the parents used in crosses 17–20 according to observed segregation ratios of the progeny (Table 3). Progeny from

Table 3. Genetic analysis of the F_2 and backcross (BC_1) generations from crosses among spring-type (S) and winter-type (W) russian dandelion plants.

Suggested model[z]	Cross no.	Cross[y]	Segregation ratio (S:W) Observed[x]	Expected	χ^{2w}	*P* value
aaBbCc × *AabbCc*	10	7-80 S × W-1	18:7	11:5	0.02	0.89
=11 *AaBbC-*, aa----:5 *Aabb--*, *AaBbcc*	11	7-13 S × W-1	42:19		0.00	1.00
AaBbcc × *AaBbcc*	12	7-24 W × W-6	9:33	1:3	0.13	0.72
=1 *aa—cc*:3 *A---cc*						
aaBbCC × *aaBbCC*	13	1-79 S × 1-3 S	72:2	1:0	—	—
=*aa—CC*	14	1-4 S × 1-52 S	20:0		—	—
AaBbCC × *aaBbCC*	15[v]	1-63 S × 1-65 S	18:1	7:1	0.37	0.54
=7 *A---CC*, *aa--CC*:1 *AabbCC*						
AaBbCc × *AaBBcc*	16	8-19 S × W-2	87:53	5:3	0.00	1.00
= 5 *A-B-C-*, *aaB---*:3 *A-B-cc*						
aabbCc × *AaBbCc*	17	7-31 S × 8-12 S	46:21	11:5	0.00	1.00
=11 *AaBbC-*, *aa----*:5 *Aabb--*, *AaBbcc*						
AABbcc × *AaBbCc*	18	7-11 W × 8-12 S	38:60	3:5	0.02	0.88
=3 *A-B-C-*:5 *A-bbC-*, *A-B-cc*						
AABbcc × *AabbCc*	19	7-11 W × 8-32 W	18:61	1:3	0.02	0.88
=1 *A-B-C-*:3 *A-B-cc*, *A-bbCc*						
AaBbcc × *AABbCc*	20	7-9 W × 8-21 S	31:54	3:5	0.01	0.93
=3 *A-B-Cc*:5 *A-bb--*, *A-B-cc*						
AaBBCc × *aaBBCc*	21	2-96 S × 2-61 S	36:3	7:1	0.44	0.51
=7 *AaBBC-*, *aaBB--*:1 *AaBBcc*	22	2-80 S × 2-86 S	35:5		0.00	1.0
	23	2-80 S × 2-73 S	26:3		0.01	0.94
AaBBCc × *AaBBCc*	24	2-78 S × 2-72 S	44:13	13:3	0.38	0.54
=13 *A-BBC-*, *aaBB--*:3 *A-BBcc*						
aaBBCc × *aaBBCc*	25	2-2 S × 2-75 S	19:0	1:0	—	—
=*aaBB–*	26	2-86 S × 2-94 S	18:0		—	—
AaBBCc or *aaBBCc* × *aaBBCC*	27	2-11 S × S-2	40:0	1:0	—	—
=*A-BBC-*, *aaBBC-* or *aaBBC-*	28	2-78 S × S-2	58:0		—	—
	29	2-1 S × S-2	17:0		—	—
aaBBCc × *AaBBcc*	30	2-43 S × W-2	17:3	3:1	0.68	0.41
=3 *AaBBCc*, *aa-bCC*:1 *AaBBcc*						
AaBbCc × *aaBbCc*	31	9-2 S × 9-3 S	53:11	25:7	0.57	0.45
=25 *AaB-C-*, *aa----*:7 *Aabb--*, *Aa—cc*						
AaBbCC × *AaBbCC* =13 *A-B-CC*:3 *A-bbCC*	32	4-66 S × 4-18 S	58:12	13:3	0.13	0.72
aaBbCc × *aaBbCc*, *aaBbCC* × *aaBbCC*, or *aaBbCC* × *aaBbCc* =*aa--C-*	33	4-31 S × 4-48 S	157:0	1:0	—	—
AaBBCc × *AaBBCc*	34	5-62 S × 5-25 S	16:3	13:3	0.01	0.97
=13 *A-BBC-*:3 *A-BBcc*	35	5-4 S × 5-2 S	61:13		0.01	0.91

[z]Genotypes proposed based on parental genotype, phenotype, and observed segregation ratios; winter-type plants have genotypes: *A-bbC-*, *A-B-cc*, and *A-bbcc* and spring-type plants have genotypes: *A-B-C-* and *aa----*.

[y]Number preceding hyphen corresponds to cross no. from which the plant originated and number following hyphen is the individual plant identifier.

[x]For some crosses, seed was collected from both parents and analyzed as two separate populations that were then pooled together after they were determined homogeneous by chi-square heterogeneity tests. Pooled results are shown.

[w]Observed segregation ratios were compared with expected ratios using the chi-square statistic adjusted with Yate's correction for continuity.

[v]Cross 15 also fits a 1:0 ratio; parental genotypes would be the same as in crosses 13 and 14.

intercrossing two spring-type plants, 7-31 and 8-12 (cross 17) segregated 11:5, suggesting the parents were *aabbCc* and *AaBbCc*, respectively. Plant 8-12 was also crossed to a winter-type plant, 7-11, and produced a 3:5 ratio in the progeny, which can be explained by crossing *AABbcc* (7-11) and *AaBbCc* (8-12) genotypes (cross 18). Thus, the proposed genotype for 8-12 was validated in two crosses and 7-11 could be *AABbcc*. Crossing 7-11 with winter-type plant, 8-32, produced 1:3 segregation, which can be explained by a *AABbcc* (7-11) × *AabbCc* (8-32) cross (cross 19). A cross between winter-type plant, 7-9, and spring-type plant, 8-21, showed progeny segregating 3:5, and can be explained by parents with genotypes *AaBbcc* and *AABbCc* (cross 20).

For crosses between spring- and winter-type plants [Table 1: S-2 × W-2 (cross 2); S-3 × W-3 (cross 3)] mostly spring-type plants were recovered and the few winter types were attributed to segregation at minor loci that did not include the *A*, *B*, and *C* loci of the proposed model; thus segregation was classified as 1:0. Based on the proposed genotypes for W-2 (*AaBBcc*) and W-3 (*AAbbCc*), S-2 and S-3 could be assigned the genotypes *aaBBCC* or *AABBCC*. Progeny therefore could be all spring type: *AaBBCc* and *aaBBCc* for the S-2 (*aaBBCC*) × W-2 (cross

2); *AaBbCC* and *AaBbCc* for the S-3 (*aaBBCC*) × W-3 (cross 3); *AABBCc* and *AaBBCc* for S-2 (*AABBCC*) × W-2 [cross 2 (genotypes not shown)]; or *AABbCC* and *AABbCc* for the S-3 (*AABBCC*) × W-3 (cross 3). Based on crosses between progeny from cross 2, the genotype *aaBBCC* was assigned to S-2 (cross 2). Progeny from cross 3 were not used in additional crosses so the exact genotype could not be defined.

Two progeny genotypes, *AaBBCc* and *aaBBCc*, were predicted for cross 2 (*aaBBCC* × *AaBBcc*). Intercrossing 10 plants that included these genotypes produced the expected segregation ratios of 7:1, 13:3, and 1:0 [Table 3 (crosses 21–26)]. Certain plants, 2-80 and 2-86, were used in two distinct crosses, and assigned genotypes that were further validated (crosses 22, 23, and 26).

Backcrosses between spring-type progeny from cross 2 and the parents, S-2 and W-2, provided further evidence for the model (Table 3). Crosses 27–29 produced all spring-type progeny that would occur by crossing either of the predicted cross 2 progeny genotypes, *AaBBCc* or *aaBBCc*, with S-2, *aaBBCC*. The observed 3:1 segregation in cross 30 can result from crossing *aaBBCc* (2-43) with *AaBBcc* (W-2).

Crossing two winter-type plants (W-4 × W-5) produced progeny segregating 94 spring- and 7 winter-type progeny [Table 1 (cross 9)]; the winter-type progeny are presumed to be caused by segregating minor genes that do not include *A*, *B*, and *C* of the proposed three-gene model and segregation of 1:0 is assumed. Recovery of only spring-type progeny from a winter-type × winter-type cross could be explained by the following parental genotypes: *AAbbCC* × *AABBcc*, *AAbbCC* × *AaBBcc*, or *AabbCC* × *AaBBcc*. Intercrossing progeny from cross 9 resulted in 25:7 segregation [Table 3 (cross 31)], which could be explained by crossing plants that are *AaBbCc* and *aaBbCc*. Consequently, the W-4 and W-5 parents are assigned *AabbCC* and *AaBBcc*, respectively, because *AABbCc*, *AaBbCc*, and *aaBbCc* progeny are produced from this cross.

W-4 (*AabbCC*) and W-5 (*AaBBcc*) were also crossed to spring-type plants S-4 or S-5, and 1:0 ratios were observed [Table 1 (crosses 4 and 5)]. Again, a number of genotypes could be assigned to S-4 and S-5 to explain the observed segregation. For example, S-4, which was crossed to W-4, could have the following genotypes: *aaBBcc*, *aaBBCc*, *aaBBCC*, *AaBBCc*, *AaBBCC*, *AABBCc*, or *AABBCC*. Progeny crosses do not provide definite proof for one genotype over another, so *aaBBCc* and *aaBBCC* are shown as examples for S-4 and S-5. S-4 (*aaBBCc*) × W-4 (*AabbCC*) produced progeny with genotypes *AaBbCc*, *AaBbCC*, *aaBbCc*, and *aaBbCC*. Intercrossing two spring-type progeny from cross 4, 4-66, and 4-18 [Table 3 (cross 32)] resulted in 13:3 segregation that could be explained by an *AaBbCC* ×*AaBbCC* cross. However, a 7:1 ratio also fits the data [$P = 0.32$ (not shown in table)], which would be produced if the parental genotypes were: *AaBbCC* × *aaBbCC*. Another intercross of spring-type progeny from cross 4 [cross 33 (4-31 × 4-48)], produced 1:0 segregation and can be explained by crossing any combination of progeny lacking a dominant *A* allele (*aaBbCc* × *aaBbCc*, *aaBbCC* × *aaBbCC*, or *aaBbCC* × *aaBbCc*).

Finally, intermating progeny from cross 5 resulted in 13:3 segregation for two matings [Table 3 (crosses 34 and 35)], which can be explained by both parents having the *AaBBCc* genotype. However, the segregation patterns for the two crosses also fit a 7:1 ratio [$P \geq 0.57$ (not shown in table)] that could be explained by crosses between *AaBBCc* and *aaBBCc* genotypes.

Discussion

Based on the segregation patterns observed in the F_1, F_2, and BC_1, at least three major genes govern flowering habit in TKS, where a dominant allele at locus *A* in combination with two recessive alleles at one of two or both loci, *B* and *C* (i.e., *A-bbC-*, *A-B-cc*, or *A-bbcc*), confers the winter type (Table 2). The spring type results from a homozygous recessive genotype for *A*, regardless of alleles at the other two loci (i.e., *aa----*), or dominant alleles at all three genes (i.e., *A-B-C-*) Although there are many segregation ratios that may fit the observed phenotypic segregation for each cross, and numerous genotypes can be assigned to each plant to produce the observed segregation, the proposed three gene model explains the data for all 38 crosses spanning three generations. Because the model is based only on the segregating loci in the germplasm studied, inclusion of other genetic materials could reveal additional, major genes controlling the trait.

Intercrossing each winter-type parent with more than one other winter-type plant would have been ideal to demonstrate that some winter-type × winter-type crosses can produce all winter-type offspring. The lack of 0:1 segregation throughout the generations suggests the genetic model may be more complex than that presented here. By examining the days to flowering for each plant in future studies rather than classifying each as either winter or spring type, additional modifying loci may be discovered. An increased number of genes controlling flowering habit, however, may also raise the effort required to produce populations that are strictly winter type and never segregate.

The self-incompatibility in TKS makes genetic analyses challenging because many crosses cannot be made due to shared alleles, and the inability to self-pollinate hinders the development of homozygous tester lines. Further analyses, mapping quantitative trait loci with molecular markers may be useful to verify and expand the genetic model, accounting for loci with major and minor effects.

The flowering habits of *A. thaliana* and wheat are controlled by multiple genes. Null mutations at the floral repressors, *FLC* and *VRN2*, respectively, determine spring type regardless of the

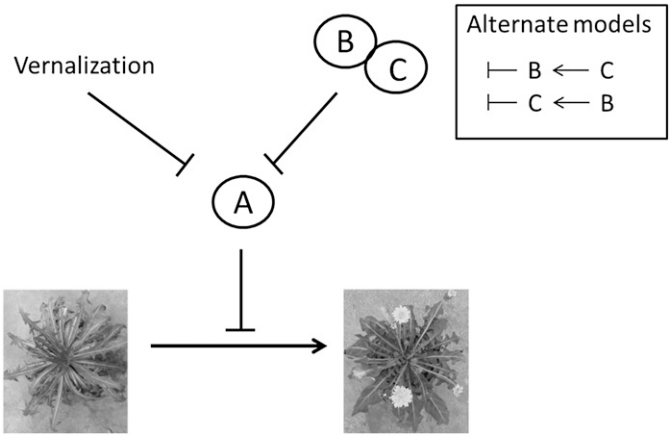

Fig. 1. A proposed model for flowering habit in russian dandelion. The protein product of gene *A* represses flowering, and vernalization or the functional products of genes *B* and *C* contribute to the repression of A. Lines ended with arrowheads and small perpendicular lines represent positive and negative regulation, respectively.

alleles at other loci. Consequently, the repressors are epistatic to the other genes. The discovery of several loci with epistatic interactions in TKS is not surprising and the model is similar to that in the cereals where winter type is determined by a dominant allele at *VRN2* in combination with homozygous recessive genotypes at both of two loci, *VRN1* and *VRN3* (Yan et al., 2004, 2006). In TKS, however, a homozygous recessive genotype at either of two loci, *B* or *C*, is sufficient to produce the winter type.

The mechanism controlling flowering habit is conserved throughout many plants, whereby a floral repressor that blocks expression of an activator of reproductive development is downregulated by vernalization. In each species where the pathway is characterized, the repressor is encoded a dominant allele (Johanson et al., 2000; Yan et al., 2004). Therefore, locus *A* in TKS is a possible candidate for the floral repressor, whereby repression can be overcome by vernalization (Fig. 1). Genes, *B* and *C*, could encode proteins responsible for repressing *A*, either together or in a pathway where one is an upstream positive regulator of the other negative regulator. In this model, homozygous recessive genotypes for any of the three genes, *aa*, *bb*, or *cc*, would produce nonfunctional proteins.

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