

Reproductive isolation of a new hybrid species, *Senecio eboracensis* Abbott & Lowe (Asteraceae)

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The nature and extent of reproductive isolation was examined between a new self-compatible hybrid species *Senecio eboracensis* ($2n=40$) and its parents, self-incompatible *S. squalidus* ($2n=20$) and self-compatible *S. vulgaris* ($2n=40$). The triploid F_1 of *S. eboracensis* × *S. squalidus* exhibited very low seed set ($\bar{x}=0.63\%$), and F_2 and F_3 progeny were able to recover nominal levels of fertility ($\bar{x}=23.9$ and 9.7%), while F_1 and F_2 offspring of *S. eboracensis* × *S. vulgaris* showed reduced seed set ($\bar{x}=63.8$ and 58.8%). In both cases, evidence from previous work indicates that reduced fertility is associated with meiotic chromosome mispairing, and is a likely consequence of recombining both parental genomes within this new taxon. No hybrid offspring between *S. eboracensis* and *S. squalidus* were found in the wild, and only one such hybrid was recorded among 769 progeny produced by *S. eboracensis*

surrounded by *S. squalidus* on an experimental plot. Natural crossing between *S. eboracensis* and *S. vulgaris* was recorded to be very low (between 0 and 1.46%) in the wild, but rose to 18.3% when individuals of *S. eboracensis* were surrounded by plants of *S. vulgaris*. It was concluded that strong breeding barriers exist between the new hybrid species and its two parents. Prezygotic isolation between *S. eboracensis* and *S. vulgaris* is likely to be largely due to both species reproducing by predominant self-fertilisation. However, differences recorded for germination, seedling survival, time of flowering and characters associated with pollinator attraction, plus significant clumping of juvenile and adult conspecifics in the wild, probably also contribute to reproductive isolation and ecological differentiation.

Heredity (2004) **92**, 386–395, advance online publication, 10 March 2004; doi:10.1038/sj.hdy.6800432

Keywords: hybridisation; polyploidy; reproductive isolation; *Senecio*; speciation

Introduction

Two major mechanisms responsible for the origin of new hybrid taxa are allopolyploidy (Winge, 1917) and recombination (Grant, 1981). Numerous studies examining the role of each of these mechanisms in the generation of hybrid species have been reported (Abbott, 1992; Soltis and Soltis, 1993, 1999; Arnold, 1997; Rieseberg, 1997; Rieseberg and Carney, 1998). Generally, polyploid hybrid lineages are isolated from parental taxa by a postzygotic isolating mechanism caused by chromosome mispairing in backcross offspring. Similarly, strong postzygotic reproductive isolating barriers often exist between homoploid recombinant hybrid species and their parents due to meiotic irregularities or the expression of sterility factors (Rieseberg *et al.*, 1995; Rieseberg *et al.*, 1996; Wolfe *et al.*, 1998a). Less well known is the nature and extent of prezygotic reproductive isolation between a hybrid species and its parents, even though such isolation is crucial to the successful establishment of a new hybrid species.

Following its origin, a polyploid or homoploid hybrid neospecies will usually occur at low frequency in

sympatry with its parents, and is likely to suffer a minority-type disadvantage if it reproduces by outcrossing (Levin, 1975). This is because most offspring will result from crosses with the parents and be sterile or exhibit low fertility (Felber, 1991; Husband, 2000). This disadvantage will be avoided if a prezygotic isolating mechanism prevents or reduces significantly the production of such offspring. Theoretical modelling shows that even relatively minor increases in autogamy, clumped distribution (McCarthy *et al.*, 1995) or ecological differentiation (Buerkle *et al.*, 2000) can favour the sympatric establishment of a new hybrid lineage. For example, in the outcrossing, homoploid hybrid species, *Helianthus anomalus* and *H. paradoxus*, prezygotic isolation is achieved from both parent taxa, *H. annuus* and *H. petiolaris*, by ecological separation (Schwarzbach *et al.*, 2001; Welch and Rieseberg, 2002), which also helps to reduce the effects of competition with the parents. In the case of *Penstemon clevelandii*, the homoploid hybrid derivative of *P. spectabilis* and *P. centranthifolius* (Wolfe *et al.*, 1998a, b), speciation was accompanied by a change of pollinator to carpenter bees, wasps and hummingbirds in the species complex (Straw, 1955, 1956). In the case of prezygotic isolation between polyploid and diploid cytotypes of the same species, field studies of *Chamerion angustifolium* have shown that the autotetraploid cytotype is prezygotically isolated from its diploid parent due to differences in flowering phenology, clumped distribution and pollinator selection (Husband and Schemske, 2000).

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Received 14 March 2003; accepted 8 January 2004; published online 10 March 2004

In the British Isles, a novel hybrid species of the native tetraploid *S. vulgaris* ($2n = 40$) and the introduced diploid *S. squalidus* ($2n = 20$) was discovered by one of the authors (RJA) in 1979 in York, England. This species was originally referred to as 'York radiate groundsel', but has recently been formally described as *S. eboracensis* (Lowe and Abbott, 2003). It is tetraploid and possesses an intermediate morphological phenotype for capitulum and leaf characters (Irwin and Abbott, 1992; Lowe, 1996). Low levels of isozyme and cpDNA variation indicate that this hybrid species probably formed on only one occasion (Irwin and Abbott, 1992; Abbott and Lowe, 1996). However, crossing studies by Lowe and Abbott (2000) indicate that hybrid derivatives similar to *S. eboracensis* can be formed relatively easily, despite strong reproductive isolating mechanisms between their parent taxa, and thus a polytopic origin cannot be ruled out.

Studies of *S. eboracensis* from several sites around York over the last 20 years have shown that it has maintained its distinctive morphological and isozyme phenotype (Irwin and Abbott, 1992; Lowe, 1996), and appears, therefore, to be reproductively isolated from both parent taxa in the field (Abbott and Lowe, 1996). Here, we report an investigation into the nature and extent of reproductive isolation between this new hybrid species and its parents. First, we investigate postzygotic barriers by examining the fertility of first- and second-generation progeny of crosses and backcrosses between *S. eboracensis* and its parents. Second, we examine prezygotic isolation by estimating levels of hybrid formation in the field and on an experimental plot, as well as differences in germination under optimal conditions, and phenology and taxon distribution at a site in the wild. During the course of field studies, an opportunity arose to monitor the relative survival of seedlings produced by *S. eboracensis* and *S. vulgaris* at the same field site. The results of this particular investigation provided information on differences between these two species in winter survival and consequently a difference in their respective ecologies.

Methods

Fertility of intertaxon crosses

Reciprocal crosses were made between *S. eboracensis* and *S. vulgaris* var. *vulgaris* using the crossing method described by Lowe and Abbott (2000). Four F_1 hybrids were raised to generate F_2 offspring (produced by selfing) and B_1 progeny. Thereafter, 204 plants, including 33 of *S. vulgaris*, 40 of *S. eboracensis*, 18 F_1 hybrids, 72 F_2 offspring and 41 reciprocal backcross offspring, were raised to maturity in a randomised block. In addition, reciprocal crosses were made between *S. eboracensis* and *S. squalidus* to generate six F_1 triploid hybrids, while another triploid hybrid was obtained naturally from a field experiment. From these triploid hybrids, nine F_2 progeny were produced through open-pollination, while backcrossing to *S. eboracensis* formed five B_1 progeny. A total of 10 F_3 plants were produced from the F_2 s following open-pollination, and backcrossing F_2 plants to *S. eboracensis* formed five backcross progeny ($B_{1,2}$). Following this, 26 B_1 , nine F_2 , five B_1 , five $B_{1,2}$ and 10 F_3 progeny were raised in a fully randomised design together with six individuals of *S. squalidus* and 11 of *S. eboracensis*. In both experiments, plants were grown in

a glasshouse under the same conditions described in Lowe and Abbott (2000).

Two fertility characters were measured on each plant at maturity. Pollen fertility was estimated as the proportion of grains stained with acetocarmine from one floret of the second capitulum to open (flower) on each plant. Seed fertility was recorded as the proportion of seeds that matured in a randomly selected capitulum left to open-pollinate in a glasshouse. In addition, for *S. eboracensis* and *S. vulgaris* plants and offspring of crosses between them, one fitness character (life-time production of capitula) was measured. Measures of fitness and fertility were subjected to one-way ANOVA, and significant differences between taxa and generations were assessed by Tukey–Kramer multiple comparisons (SAS Institute, Inc., 1990).

Phenology

The total number of flowering individuals of *S. vulgaris* and *S. eboracensis*, growing in a car park at Dalton Terrace, York (OS105 593 512), were recorded on four different dates that spanned the flowering season in 1993 (6 May, 10 June, 30 June and 30 September). In 1994, the total number of flowering individuals and the total number of open capitula per plant were recorded at the same site for both taxa on six dates (23 March, 21 April, 18 May, 7 June, 14 July and 5 August). In both years no other *Senecio* taxa grew at the site.

Germination

Fresh seed (not more than 2-weeks old) of *S. vulgaris* var. *vulgaris*, *S. eboracensis* and *S. squalidus* originally collected from plants growing in York was germinated at five temperatures (5, 10, 15, 20 and 25°C) over a period of 45 days. For each taxon, 20 seeds were placed together on 1 cm² squares of moist 3MM blotting paper. The blotting paper squares were supported by plastic beads floating in the well of a thermogradient bar partly filled with water (Horrill, 1989). For each temperature treatment, three paper squares (one for each taxon) were located in a position along the thermogradient corresponding to the temperature treatment. The thermogradient bar contained 12 wells and thus each temperature/taxon treatment was replicated 12 times. Seeds were examined each day, and those that had germinated were removed. Results for analysis were expressed by day as the cumulative proportion of seeds germinated. Data were arcsine transformed before ANOVA.

Seedling establishment

On 22 September 1994, it was discovered that the Dalton Terrace site had been completely cleared of vegetation by persons unknown. This provided an opportunity to investigate subsequent seedling establishment and survival at the site. The distinctive leaf characteristics of *S. eboracensis* (Lowe and Abbott, 2003) are expressed at the first true leaf stage allowing seedlings of this species to be easily distinguished from those of *S. vulgaris*. It was therefore possible to classify seedlings according to species type. Eight, 1 m long linear transects were set out along the edge of the site and were surveyed on 26 November 1994, and on 16 February, 5 March and 25 July 1995. During the survey period, a record was taken of the number of individuals of each taxon present (established

and new), the total number of leaves per plant (on 26 November, 16 February and 5 March only) and the number of flowering capitula per plant (on 5 March and 25 July only).

Conspecific clustering

To test whether *S. vulgaris* var. *vulgaris* and *S. eboracensis* displayed conspecific clumping at the Dalton Terrace field site, records documenting the spatial location of plants for adult populations during 1994 (14 *S. eboracensis* and 43 *S. vulgaris* plants which grew along the edge of a wall, ie only a linear distance was recorded), and for seedling establishment along the eight, one metre long transects studied during 1994/95 (27 *S. eboracensis* and 92 *S. vulgaris* seedlings/plants, again a linear distance was used to position individuals) were analysed using spatial autocorrelation statistics (Degen *et al.*, 2001). The observed Moran's Index (D (obs)) was calculated for each neighbourhood distance class using the software package SGS (by Bernd Degen, INRA Bordeaux). Positive and negative measures of the 95% confidence intervals (D (+CI) and D (-CI)) were produced based on 1000 resamplings of species types among the existing plant coordinate data. If D (obs) is greater than the D (+CI), then there is a significant autocorrelation at that distance class and therefore significant clustering of individuals by species type. Plots of Moran's Index against neighbourhood distance classes, called correlograms, were made to illustrate the level of conspecific clustering.

Hybrid formation in the wild

Up to 10 open-pollinated seeds were collected from plants of *S. vulgaris* var. *vulgaris* and *S. eboracensis* plants that were flowering at Dalton Terrace on three dates in 1993 (6 May, 10th and 30 June; a total of 966 seed from 120 *S. eboracensis* plants and 542 seed from 69 *S. vulgaris* plants), and on four dates in 1994 (21 April, 18 May, 7 June and 14 July; a total of 200 seed from 17 *S. eboracensis* plants and 797 seed from 57 *S. vulgaris* plants). Progeny were grown to maturity from these seeds and screened for hybrids between *S. eboracensis* and *S. vulgaris*. Hybrids were easily distinguished by the possession of short stubby ray florets and intermediate leaf morphology. Data from the 1993 samples were used to estimate the frequency of the radiate allele at the site, Wright's fixation index and the frequency of hybrid formation of *S. vulgaris* and *S. eboracensis* according to procedures described by Marshall and Abbott (1982).

Hybrid formation under experimental conditions of open pollination

A total of 140 individual plants of *S. squalidus* ($n=35$), *S. eboracensis* ($n=70$) and *S. vulgaris* var. *vulgaris* ($n=35$) were raised under glass in 10 cm pots. Sowing was staggered to ensure that plants flowered synchronously. Shortly before flowering, plants were placed outside in the St Andrews Botanic Garden and assigned to four different types of unit within 12 different plots. Units varied in species composition and plant arrangement. In two units, a central plant of *S. eboracensis* was surrounded by four plants of either *S. vulgaris* or *S. squalidus* arranged in a square array with a distance of 15 cm between plants. In the other two units, a central plant of

S. squalidus or *S. vulgaris* was surrounded in similar fashion by four plants of *S. eboracensis*. The 12 different plots varied in the number and type of unit contained. Four plots, denoted as 'small size plots' were composed of a single unit, with a different unit represented in each plot. In another four plots, denoted as 'medium size plots', two units of the same type were placed side by side 15 cm apart, with each plot containing a different unit type. In the remaining four plots, denoted as 'large size plots', four units of the same type were arranged 15 cm apart in a square, with each plot containing a different unit type. The 12 plots were randomised within a 4 × 3 block with plots separated from each other by a distance of 20 m.

Seed for progeny testing was sampled from central plants within units within plots on six different days (referred to as 'date' in the analysis). Plot positions within the block were rerandomised between sampling dates. Before sampling seed, one or more recently opened capitula were selected on central plant(s) within units. In single-unit plots, three capitula were selected on the central plant; in plots composed of two units, two capitula were selected on the central plant of one unit and a third capitulum was selected on the central plant of the other unit; in plots composed of four units, a single capitulum was selected on central plants in three of the units. Selected capitula were tagged and left to open-pollinate over a 3-day period. Seed set in *S. squalidus* plants surrounded by *S. eboracensis* was very low and consequently the three plots containing these units were not analysed further. From the remaining nine plots, a sample of up to 20 seed was collected from each selected capitulum for progeny analysis. In total, 2209 seed from 162 capitula were sown out, and progeny were raised to flowering under glass to examine the frequency of hybrid progeny produced by central plants within units. Hybrid progeny were identified by morphology and confirmed by isozyme analysis (Lowe, 1996). Differences in frequency of hybrid formation were examined for unit type, plot size and sampling date using a three-way interaction of a General Linear Model (Minitab Inc., release 13.32, 2000).

Results

Fertility of intertaxon crosses

Pollen fertility in F_1 progeny of crosses between *S. eboracensis* and *S. vulgaris* was significantly lower than *S. eboracensis* but not *S. vulgaris* plants, whereas seed set in F_1 progeny was significantly lower than *S. vulgaris* but not *S. eboracensis* plants (Table 1). Pollen fertility and seed set in F_2 progeny of crosses between *S. eboracensis* and *S. vulgaris* were significantly reduced relative to those of the parents (Table 1). Mean seed set in the F_2 was 58.8% relative to 82.4% for *S. vulgaris* and 78.5% for *S. eboracensis*; however, some F_2 individuals set no seed, while others exhibited very high seed set (up to 89%). Backcross progeny also exhibited a considerable range of seed set values, and the means although lower than those of the parents were not significantly different from those of *S. eboracensis* (Table 1). For pollen fertility, F_2 progeny exhibited a significantly lower mean than *S. vulgaris* and *S. eboracensis* plants (Table 1, range 62–100%) as did B_1 progeny derived from backcrosses to *S. vulgaris*. How-

Table 1 Means and standard deviations (in parentheses) of fertility and fitness traits measured on *S. eboracensis*, *S. vulgaris* var. *vulgaris* and *S. squalidus*, and offspring of intertaxon crosses and backcrosses

Taxa	<i>S. vulgaris</i> var. <i>vulgaris</i>	<i>S. eboracensis</i>	F_1	F_2 <i>S. vulgaris</i>	B_1 to <i>S.</i> <i>eboracensis</i>	B_1 to var. <i>vulgaris</i>	P
<i>Character</i>							
N =	33	40	18	72	21	20	
Pollen fertility%	97.6 ^b (3.2)	99.6 ^a (0.5)	97.0 ^{bc} (3.4)	94.5 ^{cd} (7.5)	92.1 ^d (8.1)	98.4 ^{ab} (2.5)	***
Seed set %	82.4 ^a (12.4)	78.5 ^{ab} (7.9)	63.8 ^{bc} (16.6)	58.8 ^c (26.5)	66.1 ^{abc} (36.6)	67.2 ^{bc} (18.2)	***
N =	19	11	10	40	18	10	
Total lifetime production of capitula	80.4 (26.4)	102.2 (22.8)	96.9 (35.7)	87.6 (32.1)	78.9 (30.1)	78.8 (18.9)	ns

Taxa	<i>S. squalidus</i>	<i>S. eboracensis</i>	F_1	F_2	$B_{1,2}$ to <i>S. eboracensis</i>	F_3	$B_{1,2}$ to <i>S. eboracensis</i>	P
<i>Character</i>								
N =	6	11	7	9	26	10	5	
Pollen fertility %	94.5 ^a (9.6)	77.5 ^{ab} (21.2)	36.6 (10.2) ^c	69.5 ^{ab} (35.6)	65.5 ^b (15.9)	55.4 ^{bc} (35.1)	69.1 ^b (9.2)	***
Seed set %	72.3 ^a (31.1)	74.3 ^a (24.9)	0.63 (10.8) ^c	23.9 ^b (10.4)	55.4 ^a (35.1)	9.7 ^{bc} (18.2)	13.3 ^b (9.8)	***

Significant differences (***) $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns = not significant) and results of Tukey–Kramer multiple comparison are presented. Means sharing the same superscript are not significantly different ($P \leq 0.05$).

ever, B_1 progeny derived from backcrosses to *S. eboracensis* showed a significant increase in mean pollen fertility relative to that of F_2 progeny and this mean was not significantly different from those of the parents (Table 1). No significant difference in mean lifetime production of capitula was recorded between *S. eboracensis* and *S. vulgaris* or their F_1 , F_2 or B_1 hybrid progeny.

Since *S. squalidus* is self-incompatible (Abbott and Forbes, 1993; Hiscock, 2000a, b), only open-pollinated seed was collected from parents and progeny of crosses with *S. eboracensis*. On average, triploid F_1 hybrids (verified by chromosome counts) were highly sterile and exhibited open seed set of 0.63% and pollen fertility of 36.6% (Table 1). However, fertility increased significantly in the F_2 and F_3 generations reaching 23.9 and 9.7%, respectively, for seed set, and 69.5 and 55.4%, respectively, for pollen fertility (Table 1). Fertility also increased by backcrossing F_1 and F_2 progeny to *S. eboracensis*, with mean pollen fertility reaching 65.5 and 69.1%, respectively, and mean seed set 55.4 and 13.3%, respectively.

Field phenology, germination, seedling establishment and conspecific clumping

Phenology: The number of plants flowering at Dalton Terrace on each sample date shows that *S. eboracensis* was later to flower than *S. vulgaris* in both 1993 and 1994 (Figure 1a and b). It was also evident that in 1994 there was a considerable difference between the two taxa in the time when the greatest number of capitula were open at the site (Figure 1c). In 1994, *S. eboracensis* individuals first flowered on average after 72.1 days (from the first day of the year), while *S. vulgaris* first flowered after 43.9 days. Two *S. eboracensis* × *S. vulgaris* hybrids were recorded at Dalton Terrace in 1993, but no hybrids were found at the site in 1994.

Germination: Seeds of *S. eboracensis* germinated significantly earlier than seeds of *S. vulgaris* var. *vulgaris* at low temperatures (5–15°C), while at 20°C

there was no difference, and at 25°C the reverse was true (Figure 2). Seeds of *S. eboracensis* showed a similar pattern of germination to those of *S. squalidus* between 10 and 20°C, but at 5°C were earlier to germinate and at 25°C were later to germinate.

Seedling establishment: Based on observations made along eight linear transects at Dalton Terrace in late 1994 and during 1995, it was evident that many more *S. vulgaris* than *S. eboracensis* seedlings were present at the site in November 1994, having germinated after the site had been cleared of plants in September 1994. However, by February 1995, this difference was much reduced (Figure 3a); indeed, only 48.8% of *S. vulgaris* seedlings successfully over-wintered compared to 79.2% for *S. eboracensis*. The number of individuals of both taxa present at the site continued to decline until July 1995, with very few new seedlings becoming established after winter. The ability of *S. vulgaris* to over-winter appeared to be correlated with the number of leaves it produced before winter. Those *S. vulgaris* plants that successfully over-wintered (ie were still alive in February) had significantly more leaves in November 1994 (*t*-test, $P < 0.001$; mean number leaves = 7.53, $n = 40$) relative to plants that died (mean number of leaves = 5.94, $n = 49$). This was not the case for *S. eboracensis* individuals (mean number of leaves on plants that survived winter = 6.86, $n = 18$; mean number of leaves on plants that died during winter = 6.94, $n = 7$). It was also evident from the number of capitula recorded per plant in March and July 1995 that *S. vulgaris* again flowered earlier than *S. eboracensis* (Figure 3b).

Conspecific clumping: The spatial distribution of adult plants of *S. vulgaris* and *S. eboracensis* at the Dalton Terrace site during 1994 showed significant clumping of conspecifics (Figure 4a, the observed Moran's Index of autocorrelation exceeds the upper 95% confidence limit). Clumps tended to be approximately 2–4 m across. Significant spatial autocorrelation was also found in the distribution of juveniles of the two species along the seedling establishment transects at the same site during

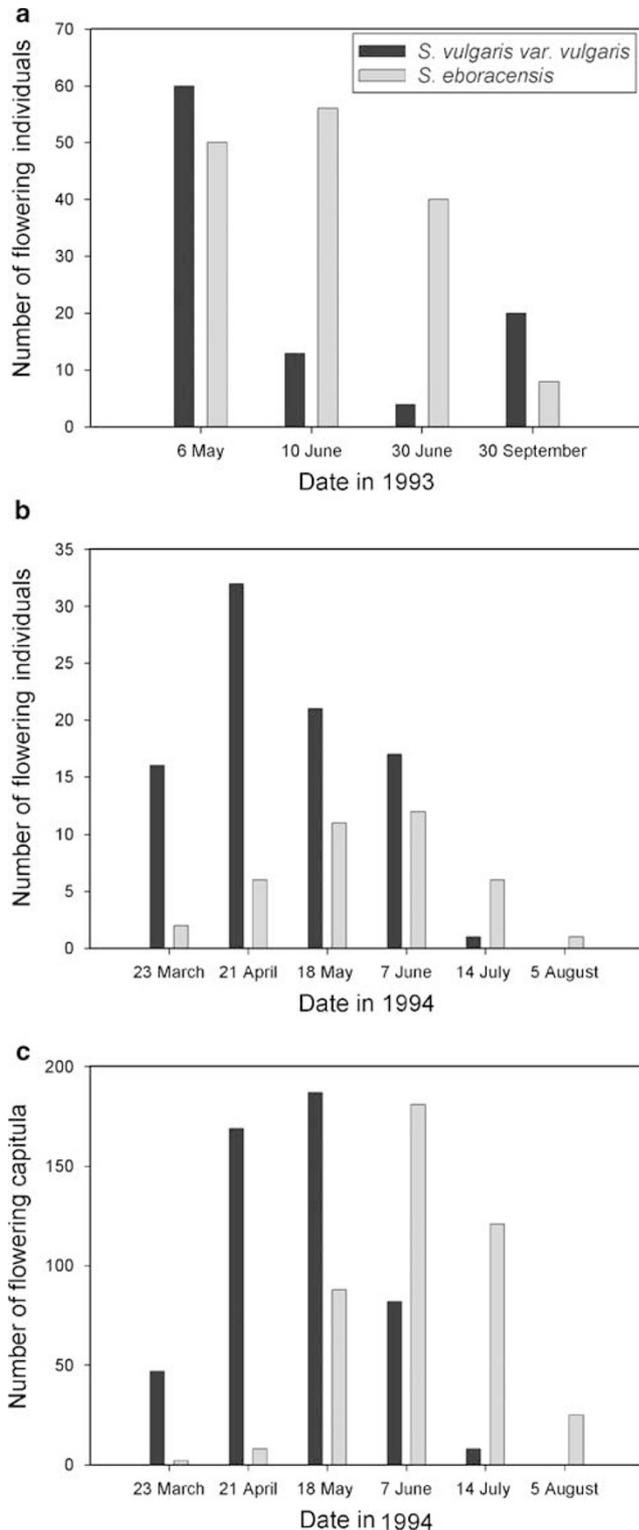


Figure 1 Number of individuals of each taxon flowering on a specified date at Dalton Terrace during (a) 1993 and (b) 1994. (c) Total number of flowering capitula produced by all plants of each taxa on a specified date at Dalton Terrace during 1994.

1994/1995. Although these eight transects were only 1 m long, almost all distance classes up to 1 m exhibit a significant effect of conspecific juvenile clumping (Figure 4b).

Hybrid formation in the wild and under experimental conditions of open-pollination

In the wild: In 1993, the frequency of the radiate allele (Tr) in the population of flowering plants at Dalton Terrace increased markedly from early May to late June (0.389–0.909, Table 2). This reflected the later flowering time of *S. eboracensis*. The high values recorded for Wright's fixation index (>0.95) indicate that levels of heterozygosity at the ray floret locus, and therefore hybridisation between *S. eboracensis* and *S. vulgaris*, were low. Progeny tests showed that the rate of hybrid formation by maternal plants was extremely low for *S. eboracensis* ($t_{(TrTr)}=0-0.0146$) and was zero for *S. vulgaris* var. *vulgaris*. In 1994, progeny tests indicated that no hybrids were produced at Dalton Terrace.

Experimental conditions: Differences between the three different types of unit employed in the experiment explained the highest proportion of variation in the GLM of frequency of hybrid formation and was highly significant ($P<0.001$, Table 3). Plot size (in terms of number of units per plot) and sampling date did not significantly influence levels of hybrid formation and there was no significant interaction between variables. For data pooled over different sampling dates (Table 4), the mean frequency of hybrid formation was highest for units in which *S. eboracensis* was surrounded by *S. vulgaris* var. *vulgaris* (18.3%), and much lower in units with the reciprocal planting design (1.5%). Only one triploid F_1 hybrid was raised from seed collected from units in which *S. eboracensis* was surrounded by *S. squalidus* plants yielding a mean frequency of hybrid formation equal to 0.1%.

Discussion

It is clear from the low levels of interspecific hybridisation recorded in the wild and also under experimental conditions that the new hybrid species *S. eboracensis* is reproductively isolated from its parents, *S. vulgaris* and *S. squalidus*. In part, this is due to a strong prezygotic isolating barrier between *S. eboracensis* and *S. vulgaris* largely due to the predominant selfing of both species. Each species is self-compatible and sets high levels of seed when selfed (*S. eboracensis* – 78.8%; *S. vulgaris* – 82.4%, Table 1). In addition, *S. eboracensis* is strongly isolated from *S. squalidus* by a postzygotic barrier due to a difference in ploidy, and less strongly from *S. vulgaris* by reductions in seed and pollen fertility. *S. eboracensis* was also found to exhibit greater winter survival of seedlings relative to *S. vulgaris*, indicating that these two species are ecologically differentiated to some extent and therefore likely to fill different ecological niches.

Fitness of hybrids and frequency of hybridisation between *S. eboracensis* and its parents *S. squalidus* and *S. vulgaris*

As expected, a difference in ploidy between *S. eboracensis* ($2n=40$) and *S. squalidus* ($2n=20$) creates a very strong postzygotic breeding barrier between these two species. First-generation hybrids are triploid and highly sterile (mean seed set equals 0.63%). On average, two trivalents, and from four to eight univalents were produced in meiotic cells of one hybrid examined, demonstrating a

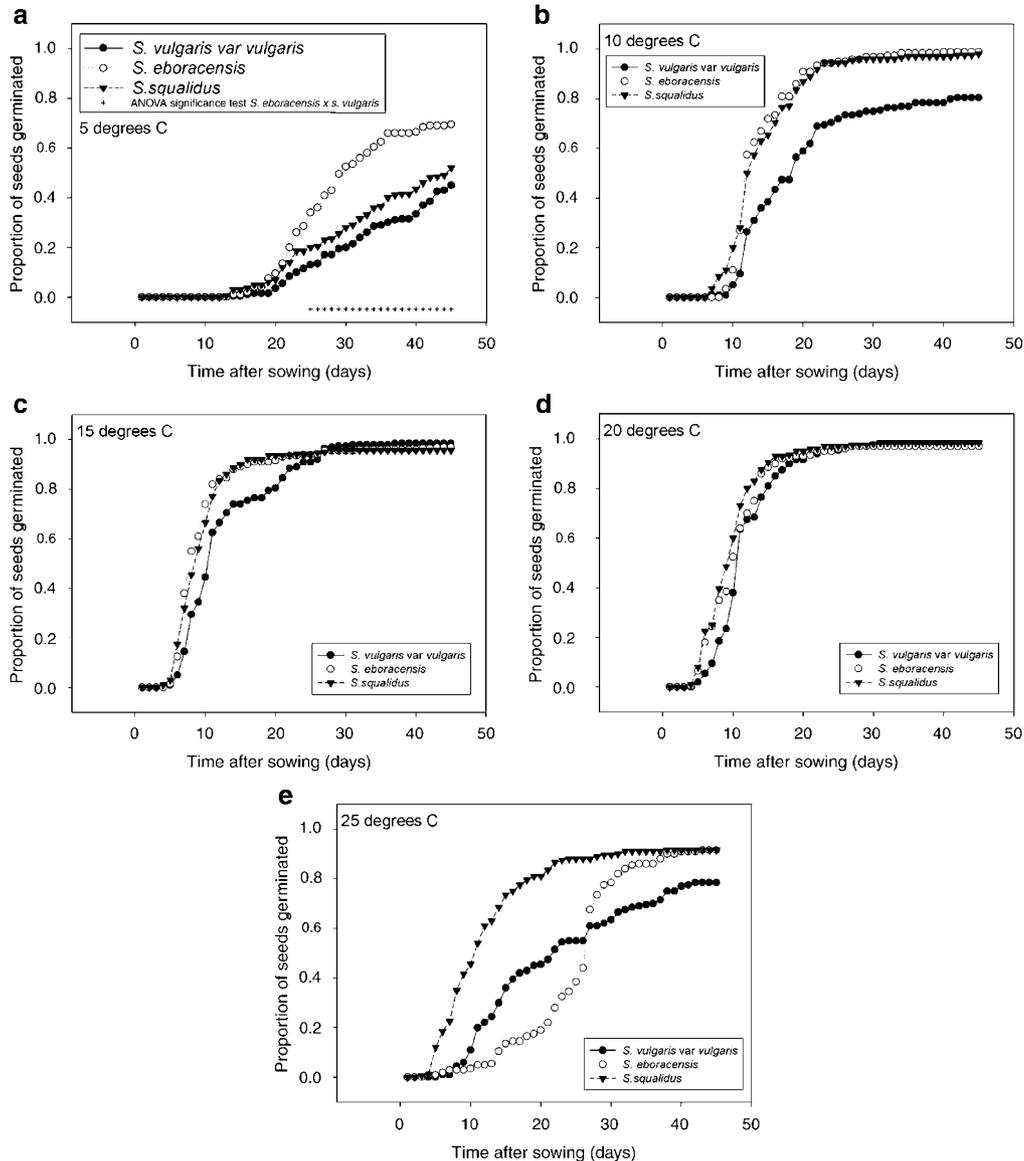


Figure 2 Cumulative proportion of seeds germinated over time at (a) 5°C; (b) 10°C; (c) 15°C; (d) 20°C; (e) 25°C. Proportion of seeds germinated on each day were arcsine transformed and subjected to ANOVA, crosses indicate that there was a significant difference ($P < 0.05$) in the proportion of seeds germinated between *S. vulgaris* var. *vulgaris* and *S. eboracensis*.

high level of meiotic irregularity (Lowe, 1996). Later (F_2 and F_3) and backcross (B_1 and $B_{1,2}$) generations of this cross exhibited a wide range of fertilities, but in general fertility was higher than the F_1 generation indicating that a resumption in fertility, probably through genome stabilisation, may be possible for such products.

Hybridisation between *S. eboracensis* and *S. squalidus* is a rare event. No hybrids between these two species were recorded in the field, and the level of hybridisation was estimated to be only 0.1% when *S. eboracensis* plants were surrounded by *S. squalidus* on an experimental plot in the Botanic Gardens at St Andrews.

F_1 progeny of crosses between *S. eboracensis* and *S. vulgaris* exhibited significantly reduced mean seed set (63.8%) compared to *S. vulgaris* (82.4%) and significantly reduced pollen fertility (97%) compared to *S. eboracensis* (99.6%). Rather than recovering fertility,

second-generation products of this cross exhibited a further reduction in mean seed set (58.8%) compared to the parents (78.5 and 82.4%, respectively, $P < 0.001$), although a wide range of seed fertilities were observed in the F_2 . For one F_2 plant whose meiosis was examined, two univalents were observed indicating a lack of homology between one pair of chromosomes (Lowe, 1996). Backcross progeny demonstrated a recovery in seed fertility (66.1 and 67.2%, respectively, for B_1 crosses to *S. eboracensis* and *S. vulgaris*, respectively), compared to F_1 (63.8%) and F_2 (58.8%) generations. The observed fluctuation in fertilities, particularly the wide range of variation observed in later generation products, is consistent with a genome stabilisation process. However, these results indicate that *S. eboracensis* must contain a substantial portion of the *S. squalidus* genome, or major structural rearrangement of the *S. vulgaris* genome, to

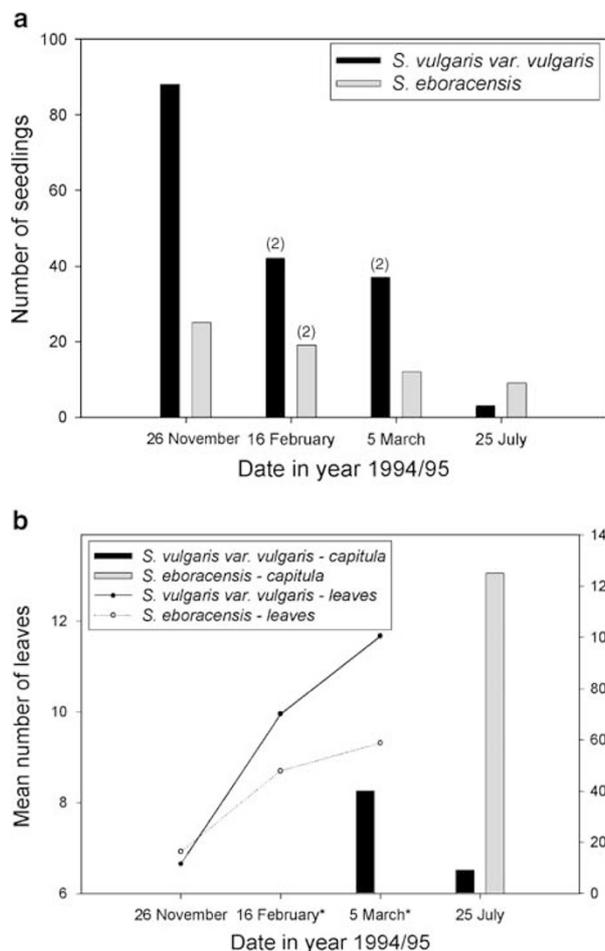


Figure 3 (a) Total number of seedlings of each taxon recorded on specific dates over the winter season 1994/95 at Dalton Terrace. The number of newly established seedlings is indicated in parentheses above bars. (b) Mean number of leaves per plant and total number of flowering capitula produced by all plants of each taxon between November 1994 and July 1995. Asterisks by date indicate significant difference ($P < 0.05$) in the mean number of leaves per plant between taxa.

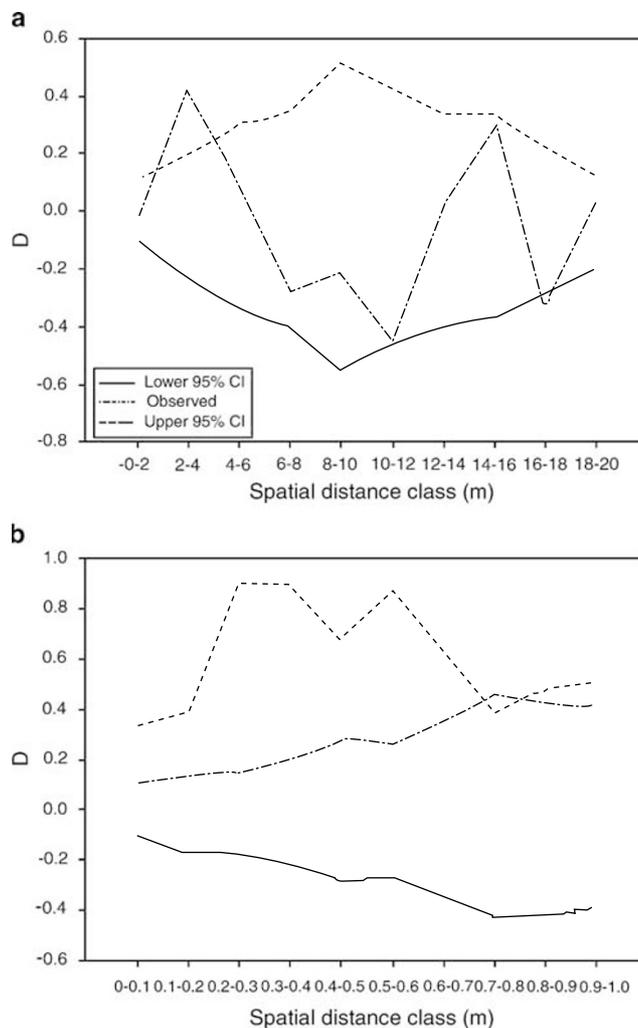


Figure 4 Test of spatial clustering of *S. vulgaris* and *S. eboracensis* plants at Dalton Terrace field site. Observed Moran's Index and 95% confidence intervals plotted against spatial distance class for (a) flowering plant data from 1994 and (b) for seedling establishment plots from 1994/95.

Table 2 Number of flowering plants of each taxon and their hybrid at Dalton Terrace in 1993.

Date	6.5.1993	10.6.1993	30.6.1993	30.9.1993
Flowering plants				
<i>S. eboracensis</i>	36	56	40	8
<i>S. vulgaris var. vulgaris</i>	57	13	4	20
Hybrid	2	1	0	0
<i>S. eboracensis</i> (<i>TrTr</i>)	0.379	0.800	0.909	0.286
<i>S. vulgaris</i> (<i>TnTn</i>)	0.600	0.186	0.091	0.714
Hybrid (<i>TrTn</i>)	0.021	0.014	0	0
<i>Tr</i>	0.389 (0.239)	0.807 (0.535)	0.909 (0.406)	0.286 (0.007)
<i>F</i>	0.956 (0.138)	0.954 (0.080)	1.000	1.000
Hybrid and total progeny scored				
<i>S. eboracensis</i>	2/223 (36)	1/475 (50)	0/268 (34)	—
<i>S. vulgaris var. vulgaris</i>	0/396 (57)	0/127 (9)	0/19 (3)	—
$t_{(TrTr)}$	0.0146 (0.009)	0.0109 (0.012)	0	—
$t_{(TnTn)}$	0	0	0	—

Genotype frequencies at the ray floret locus (*TrTr* – radiate, *S. eboracensis*; *TrTn* – short rayed, hybrid; *TnTn* – nonradiate, *S. vulgaris*), Wright's Fixation Index (*F*), and the frequency of the radiate allele (*Tr*) at the site. Number of hybrids over total progeny scored (with number of maternal plants shown in parentheses) are also presented, plus the frequency of hybrid formation *t* by maternal plants. Standard deviations are given in parentheses for *F*, *Tr* and $t_{(Tr,Tr)}$

Table 3 Results of a GLM analysis on the frequency of hybrids produced by central plants in three different types of unit (*S. eboracensis* surrounded by *S. vulgaris*; *S. eboracensis* surrounded by *S. squalidus*, and *S. vulgaris* surrounded by *S. eboracensis* plants)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Unit type (ut)	2	1.10258	1.10258	0.55129	43.35	0.000
Plot size (ps)	2	0.04250	0.04250	0.02125	1.67	0.193
Sampling date (d)	5	0.09612	0.09612	0.01922	1.51	0.192
ut × ps	4	0.04503	0.04503	0.01126	0.89	0.476
ut × d	10	0.22292	0.22292	0.02229	1.75	0.078
ps × d	10	0.03358	0.03358	0.00336	0.26	0.988
ut × ps × d	20	0.07815	0.07815	0.00391	0.31	0.998
Error	108	1.37355	1.37355	0.01272		
Total	161	2.99443				

Table 4 Percentage of hybrids produced for unit types in plots of different size after pooling data over sampling dates

Unit type	<i>S. eboracensis</i> <i>S. squalidus</i>	<i>S. eboracensis</i> <i>S. vulgaris</i> var. <i>vulgaris</i>	<i>S. vulgaris</i> var. <i>vulgaris</i> <i>S. eboracensis</i>	
Central plant				
Surround plants				
No. progeny tested	714	733	762	
Mean % hybrids produced				
Small (single-unit plots)	0.3	13.6	0.3	
Medium (two-unit plots)	0	18.1	1.2	
Large (four-unit plots)	0	23.1	3.0	
Mean	0.10 (0.10) ^b	18.26 (2.46) ^a	1.51 (0.73) ^b	<i>P</i> ***

Means for unit type (over plots of different size) are significantly different ($P \leq 0.05$) if they do not share the same superscript. Standard deviations are shown in parentheses.

cause significant fertility consequences when backcrossed to the parent with which it shares ploidy and much morphological similarity.

Hybridisation between *S. eboracensis* and *S. vulgaris* also occurs rarely in the wild. Two mature hybrids between these species were recorded at the Dalton Terrace site in 1993, and none was found there in 1994. Progeny tests of *S. eboracensis* offspring produced at this site yielded estimates of intertaxon crossing of 1.46%, 1.09% and zero, respectively, on three different sampling dates during the main flowering period in May and June 1993. However, no hybrids were recorded among seeds sampled from *S. vulgaris* on the same dates. In addition, no hybrids were present among 997 *S. eboracensis* and *S. vulgaris* offspring produced at the site in 1994.

Rates of hybridisation will depend to some extent on the spatial arrangement of the species at a given site. Thus, when *S. eboracensis* was surrounded by plants of *S. vulgaris* on the experimental plot (conditions that would promote gene flow between the species), the frequency of hybrids among its offspring rose to 18.3%. In contrast, the rate of hybridisation fell to 1.4% when *S. vulgaris* was surrounded by *S. eboracensis*. This difference may be partly or wholly explained by the fact that *S. eboracensis* produces radiate capitula comprised of a mix of ray and disc florets. Ray florets are male sterile and open first within a capitulum to promote outcrossing (Marshall and Abbott, 1984). In contrast, *S. vulgaris* produces nonradiate capitula composed entirely of hermaphrodite disc florets that are known to show very low levels of outcrossing (Marshall and Abbott, 1984). There may also be a pollinator-mediated effect contributing to differences in rates of hybrid formation. *S. eboracensis* plants are much more conspicuous to the eye than *S. vulgaris* due to the possession of ray florets,

and also produce more stigmatic papillae, which can promote the capture of incoming pollen (Richards, 1986). In addition, *S. eboracensis* produces more pollen grains per floret than *S. vulgaris* (Lowe, 1996), and pollen is a major food source of hoverflies, the most common pollinator of ragwort and groundsel (Gilbert, 1986). Abbott and Irwin (1988) showed that nonradiate capitula of *S. vulgaris* are less attractive to pollinators relative to radiate capitula. Thus, pollinator transition flights and associated pollen flow from *S. vulgaris* to *S. eboracensis* may be more common than transition flights and pollen flow in the reverse direction. This could boost the maternal intertaxon crossing rate of *S. eboracensis* relative to that of *S. vulgaris*.

One potential complicating factor of such a crossing bias is a cytoplasmic/maternal interaction. Previous work on cpDNA variation and resynthesis studies indicate that *S. vulgaris* most probably acted as the maternal parent during the formation of *S. eboracensis* (Abbott and Lowe, 1996; Lowe and Abbott, 2000). Based upon these results, both taxa share similar if not identical cytoplasmic genomes and such a bias may be ruled out, although further work on this issue may be advisable.

Ecological differentiation (including phenology) and taxon distribution

At Dalton Terrace, *S. vulgaris* plants flowered mainly from April to June, whereas *S. eboracensis* plants flowered mainly between May and July in both 1993 and 1994. *S. eboracensis* was also observed to flower later than *S. vulgaris* in 1995. *S. vulgaris* seeds germinated more rapidly than those of *S. eboracensis* at high temperatures, and field observations showed that *S. vulgaris* seedlings developed more quickly in early spring relative to those

of *S. eboracensis*. Both observations would promote earlier flowering in *S. vulgaris* individuals produced from seeds that were shed in late summer and which germinated during early autumn. Although phenological separation is an important prezygotic isolating mechanism between related species (Levin, 1978, 2000), it is probably only a contributing mechanism in the case of *S. eboracensis* and *S. vulgaris* as low levels of intertaxon crossing were recorded during periods when both species were flowering synchronously (May and June).

Another factor that could significantly reduce the level of intertaxon crossing in the wild is conspecific clumping of *S. vulgaris* and *S. eboracensis* plants, which was recorded at the Dalton Terrace site. Given the expected greater attractiveness of *S. eboracensis* to pollinators, pollinator visitation is likely to be more prevalent within and between clumps of this species, rather than between clumps of the two species.

S. eboracensis co-occurs with *S. vulgaris* in the wild, and might be expected therefore to have a very similar ecology. However, seedling survival over winter was proportionally much lower for *S. vulgaris* than *S. eboracensis*. There was a significant positive correlation between the number of leaves present before the winter period and the likelihood of winter survival for *S. vulgaris* (ie larger plants are more likely to survive), but not for *S. eboracensis*. Slug tracks and damage to plants were noted during all field visits. The slug, *Deroceras reticulatum*, is known to preferentially feed on groundsel plants, causing mortality of smaller individuals. A study of mollusc grazing by Warren (1987) found that seedling mortality was significantly higher for *S. vulgaris* than for radiate groundsel plants from York, and the latter in turn were preferentially grazed compared to *S. squalidus*. These observations indicate niche separation between the taxa.

Expectations of reproductive isolating mechanisms from modelling studies

McCarthy *et al* (1995) have shown that homoploid hybrid speciation is most likely to occur if the hybrid is predominantly autogamous. More recently, Buerkle *et al* (2000) showed that in cases where a hybrid reproduces by outcrossing, ecological and spatial isolation were crucial to the establishment of a new homoploid hybrid species. Husband and Schemske (2000) have further demonstrated that the establishment of an autopolyploid in sympatry with its diploid parent will be greatly enhanced by assortative mating caused by asynchronous flowering and pollinator preference. In addition, several studies (McCarthy *et al*, 1995; Husband and Schemske, 2000) have identified a clumped distribution as an important factor to reduce intertype pollinations.

In the current study, crosses between *S. eboracensis* and both parent taxa produce progeny of reduced fertility. In addition, *S. eboracensis* appears to be predominantly autogamous and the level of interbreeding with parent taxa is further reduced in the field by phenological differences, pollinator preference and a clumped distribution. There are probably also some ecological tolerance differences between *S. eboracensis* and its parents, which could further reduce intertaxon crossing and competition effects.

It is clear, therefore, that a combination of several characters promotes pre- and post zygotic reproductive isolation between *S. eboracensis* and its parents, and will have helped the establishment of this new species during the period following its origin (approximately 30–50 years ago), that is when its numbers remained low (Lowe and Abbott, 2003). Thus the new species will have been subject to a much reduced level of minority type disadvantage during its establishment phase. It is likely that the suite of characters causing reproductive isolation was present in the neospecies when it first originated. However, the relative roles of selection and stochastic effects in the formation of this suite of characters in the new hybrid remain to be assessed.

Acknowledgements

We are grateful to David Forbes for his assistance in the greenhouse, to Ron Smith for advice with statistical methods and to Stephen Harris for constructive comments on an early version of the manuscript. The research was conducted while AJL held a research studentship from the NERC.

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