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Evaluating the role of ecological isolation in maintaining the species boundary between *Silene dioica* and *S. latifolia*

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Abstract The relative importance of floral versus ecological isolation in preventing introgression remains unclear. This study examines whether ecological isolation can explain the continuing integrity of *Silene dioica* and *S. latifolia* where floral isolation is weak and hybrids are fully viable. Eighteen small replicate founder populations of 6 individuals (3 males and 3 females) of either *S. latifolia*, *S. dioica* or hybrids were created in woodland and in open sites in southern UK. Survival, reproduction and introgression of these populations were examined over 9 years. *S. latifolia* and hybrid plants suffered higher mortality than *S. dioica* in woodland. In open sites, there was extensive introgression, with few or no pure *S. latifolia* or *S. dioica* surviving by the end of the experiment. The experiment suggests that the integrity of *S. dioica* is maintained by its ability to survive in shaded habitats where *S. latifolia* and hybrids cannot persist. However, how *S. latifolia* survives as a distinct species in the study area remains a puzzle. Immigration from regions where *S. latifolia* occurs in isolation (i.e. large-scale ecological isolation) may balance introgression in the study area.

Keywords Hybrids · Introgression · Pollination · Survival · Seed predation

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Introduction

The relative importance of floral versus ecological isolation in promoting speciation and preventing introgression in angiosperms remains unclear, and has stimulated classic studies of hybridization in *Aquilegia* and *Ipomopsis* (Grant 1952, 1992, 1993a, b; Chase and Raven 1975; Hodges and Arnold 1994; Campbell 2004). Where there are no internal (post-mating) barriers to hybridization and the species concerned are sympatric, species integrity must be maintained by either floral, phenological or ecological isolation (Grant 1993a). Floral isolation occurs when two plant species have differing flower morphology, which mechanically reduces pollen transfer between them, or when foraging strategies of pollinators result in little interspecific pollination. These two factors commonly interact, since pollinator visitation and foraging strategy depend upon floral morphology (Lewis 1993; Grant 1994). Many pollinating insects exhibit flower constancy; individual insects learn to associate a particular colour, shape or scent with reward and visit a particular flower morph to the exclusion of others (Darwin 1876; Goulson et al. 1997; Goulson and Cory 1993; Goulson and Wright 1998). Flower constancy may contribute to floral isolation as it could result in assortative mating among sympatric species of plants which differ in floral morphology (Grant 1994; Goulson 1994; Hodges et al. 2004). Whilst floral isolation has been extensively studied, the importance of ecological isolation has received less attention, perhaps because the mechanism is obvious: if two species occur in different habitats, gene flow between them will inevitable be reduced. Ecological isolation may occur through genetic differences in habitat preferences such as altitude (Ramsey et al. 2003; James and Abbott 2005; Kimball 2008), and is susceptible to anthropogenic habitat disturbance (Bleeker and Hurka 2001). There is good evidence that ecological isolation from the parental species is vital for the persistence of hybrid plant species (Rieseberg 1997; Buerkle et al. 2000; Ma et al. 2006), and artificial transportation of hybrids to locations where the parental species are absent can allow them to persist indefinitely (James and Abbott 2005).

Although floral and ecological isolation have been examined in depth in some systems, few generalizations can be made and further studies of different systems are needed to determine their relative importance. Here, I examine introgression between *Silene latifolia* Poiret and *S. dioica* (L.) Clairv. (white and red campion, respectively, Caryophyllaceae). *Silene* species have been the subject of numerous ecological, genetic and evolutionary studies spanning many decades, and therefore, their biology is better known than that of most wild plants.

Both *S. latifolia* and *S. dioica* are abundant in southern England but occupy different habitats (Baker 1947). *S. latifolia* occurs as a biennial or perennial in open disturbed habitats such as fallow fields and field margins, and is particularly abundant on chalk soils (it also occurs in scrub and woodland in the Mediterranean) (Baker 1947). *S. dioica* is a perennial associated with woodland, woodland edge and hedgerows, preferring moister soil than *S. latifolia* and being more tolerant of shade (Baker 1947, 1948a; Willmot and Moore 1973; Prentice 1979; Karrenberg and Favre 2008). Across Europe, the two species are widespread and have broadly overlapping ranges within which introgression is frequent (Minder and Widmer 2008; Prentice et al. 2008; Karrenberg and Favre 2008). *S. latifolia* and *S. dioica* can be discriminated from each other reliably using a combination of seed, flower and capsule characters (Baker 1948b; Prentice 1979; Minder et al. 2007). The species are frequently parapatric where their habitats adjoin and are sympatric in hedgerows and former woodland, and in both situations, viable hybrids occur (Baker 1948a). These exhibit intermediate morphology often including pink flowers (although this is not a reliable character for identifying hybrids). Identification of hybrids requires the use of several morphological characters in conjunction (Baker 1947), although even then some hybrid plants may be indistinguishable from the parental species (Nigtevecht 1966; Minder et al. 2007).

The floral structure of *S. latifolia* and *S. dioica* is similar and flowering phenology shows substantial overlap (particularly between male *S. dioica* and male and female *S. latifolia*). It has long been known that there are differences in pollinator guilds between the two species, with *S. latifolia* pollinated primarily by moths, and *S. dioica* by bumblebees and butterflies (e.g. Crie 1884; Knuth 1898). However, moths, butterflies, bumblebees and hoverflies have been recorded visiting both species (Knuth 1898; Baker 1947; Goulson and Jerrim 1997). When grown nearby, pollinator movement and transfer of fluorescent powder pollen analogues between species is frequent (Goulson and Jerrim 1997; van Putten et al. 2007). *S. latifolia* and *S. dioica* have the same chromosome number ($2n = 24$) and probably evolved from a recent common ancestor (see Prentice 1979 for a discussion). They can be artificially crossed to give F1 hybrids capable of intercrossing or backcrossing with either parent with no apparent loss of fertility (Baker 1947; Clapham et al. 1987), although there may be a female biased sex-ratio in the F1 generation (Taylor 1994). Hybrids produce a larger mass and greater number of viable seeds than either of the parental species (Goulson and Jerrim 1997). Hence, there is no known postmating barrier to introgression and little floral isolation. Both molecular and allozyme markers suggest that there are high levels of introgression between species in areas where they are sympatric (Goulson and Jerrim 1997; Minder et al. 2007; Minder and Widmer 2008), yet the two parental species persist and molecular studies confirm that they should be regarded as distinct species (Minder and Widmer 2008; Karrenberg and Favre 2008). By default, it seems probable that species integrity is maintained by ecological isolation and differential selection according to habitat (Goulson and Jerrim 1997), but it is not clear how this operates, given that the two species occupy overlapping sets of habitats and freely hybridise. The relative fitness of the two species and hybrids in natural situations has never been examined.

The aim of this study was to examine the effects of habitat (woodland vs. open sites) on the long-term population dynamics of small founder populations of *S. dioica*, *S. latifolia* and F1 hybrids over 9 years. By translocating species from the habitat in which they typically occur, I examine how habitat affects survival, reproduction and the rate of introgression between species. I predict that survival and/or reproduction of parental types should be lower in the habitat in which they do not naturally occur, and that of hybrids should be lower than either of the parent species when the latter is growing in their natural habitat.

Methods

During April 1995, 40 plants (ten plants of each sex of both species) were transplanted from wild populations in Hampshire, UK, into 90-mm-diameter flower pots and grown in an unheated glasshouse in Southampton until they flowered. The *S. dioica* plants were obtained from 3 populations in deciduous woodland, and the *S. latifolia* plants from 5 populations in arable field margins. Given the frequency of hybrid swarms in this area (Goulson and Jerrim 1997), all populations are likely to have been exposed to some degree of introgression in the past. Species were identified following the criteria described by Baker (1947), and using a combination of flower colour, capsule shape, calyx shape and growth form. Of these, length of the calyx teeth is thought to be most reliable (*S. latifolia* males 4.8 ± 0.3 mm, *S. latifolia* females 6.5 ± 0.1 mm, *S. dioica* males 2.2 ± 0.3 mm and *S. dioica* females 2.3 ± 0.3 mm, mean ± SD, from Baker 1947). Only plants which could be clearly assigned as *S. dioica*, *S. latifolia* were used. Male and female plants were kept in separate glasshouses, and insects were excluded from both. As female flowers became available, pollen from randomly chosen male flowers was transferred to the stigma using a match stick. The female flower was then sealed within a labelled glassine bag, and seeds were subsequently collected. Each male plant was used to fertilize only one flower per female plant, and seeds from each capsule were reared in separate seed trays. One plant per seed tray was potted on. Pure *S. latifolia, S. dioica* and F1 hybrids from these crosses were then randomly selected and planted into field sites in September 1996. This protocol ensures that none of the experiment plants were full siblings, but some would have shared either the male or the female parent.

Each experimental population was founded by planting three males and three females in a 1 m^2 area, simulating a small founder population as might naturally occur. In previous studies in the area, Goulson and Jerrim (1997) mapped local populations of *Silene* and 27% of populations recorded consisted of 6 or fewer plants, and so this population size is representative of natural populations. Three founder populations each of *S. latifolia, S. dioica* and F1 hybrids were planted in both an open site and in deciduous woodland near Chilworth, Hampshire (18 populations in total). F1 hybrids consisted of mixed ♂ *S. dioica* × ♀ *S. latifolia* and ♂ *S. latifolia* × ♀ *S. dioica*. Soil type was a neutral loam. At the open site, the soil was tilled prior to planting. The woodland site was dominated by *Quercus robur* with little herbaceous cover beneath, and was not tilled prior to planting. Within each site, the populations were planted at least 20 m apart (populations of the two species are frequently found in closer proximity in natural situations). The woodland and open sites were approximately 700 m apart. Once planting was complete, the sites were not subsequently managed, the open site thus simulating conditions in arable set-aside. No wild *Silene* could be found within 300 m of the experimental populations, but both parental species and hybrids occur naturally within 1 km.

Populations were monitored on 8–14 June each year until 2004. All plants were recorded within a 3 × 3 m area centered on the original 1 m^2 in which the founding plants were placed. Where plants were in flower, the species, sex and number of flowers were recorded. Hybrids were identified using the combination of characters described above. Molecular markers have recently been developed that would probably have been more accurate (Minder et al. 2007; Karrenberg and Favre 2008), but these were not available when this study commenced, and the correlation between morphological and genetic markers for hybrid identification is high (correlation coefficient = 0.71–0.77) (Minder et al. 2007).

Cases of anther-smut (*Microbotryum violaceum*) were noted. All capsules on female plants were examined to record damage by lepidopteran larvae. A sample of 30 larvae were removed in 1997 and reared in captivity to establish their identity. In 1999, one damaged and one undamaged seed capsule per female plant was randomly sampled (this was only done at open sites since no seed predation was recorded in woodland). Seeds remaining per capsule were counted.

In the winter of 2004, approximately 7½ years after the experimental population was set up, the open sites were ploughed and harrowed, to simulate the occasional cultivation that would be expected in an agricultural environment.

Statistical analysis

All ANOVAs were performed in SPSS16.0. Following checks for homogeneity of variances, population size over time was examined using repeated measures ANOVA with each experimental population as the unit of replication (with the number of plants within the population as the dependent variable), year as the repeated measure (within-subjects variable) and plant species and habitat as between-subject variables. By default, SPSS also examines whether there are significant higher order interactions (quadratic, cubic etc.) between the dependent variable and the within-subjects variable (year). The number of flowers per plant was examined using three-way ANOVA according to plant species, sex and habitat (fixed factors) using pooled data for all populations and years (having confirmed that there was no significant variation between years, $F_{7,436} = 1.58$, n.s.), with plant as the unit of replication. Tukey's post hoc tests were used to examine differences between species. The proportion of hybrids within populations which started out as pure *S. dioica* or *S. latifolia* was examined using repeated measures ANOVA on arcsine transformed data, with species and habitat as fixed factors. The proportion of capsules that were predated on each female plant was analysed using plant as the unit of replication following arc-sine transformation, with plant species, year and habitat as fixed factors (independent variables). Seed set per capsule was examined using two-way ANOVA with damaged/undamaged and plant species as fixed factors. In all cases, residuals were normally distributed. Differences in sex ratio among *S. dioica, S. latifolia* and hybrid plants were examined using a χ^2 test of association on data pooled for all populations and years.

Results

Population changes over time

Population trends differed markedly between woodland and open sites. In woodland, all *S. latifolia* and hybrid founder populations died within 6 years (Fig. 1). In contrast, two of the three *S. dioica* founder populations persisted for the 9 years of the experiment, although there was little recruitment. In open sites, most founder populations fared better, at least in the short term, with considerable recruitment (Fig. 1). It must be noted that these measures of population size include all species present (and hybrids). Repeated measures analysis of variance on population size according to starting species and habitat revealed a significant three way interaction among date, habitat and species, indicating that all three factors have a significant effect (Table 1). Mauchly's test of sphericity was significant ($\chi^2_{35} = 112$, $P < 0.001$), but the result remains unchanged after adjusting the degrees of freedom accordingly (Table 1). There was also a strong quadratic relationship between time and population size, due to a tendency for populations in open sites to initially increase and later to decline ($F_{1,12} = 43.1$, $P < 0.001$) (Fig. 1). It is worth noting that variation within treatments was high; for example, two of the three hybrid populations established in open sites went extinct, while the third flourished, growing to contain over 50 individual plants by the end of the experiment. This variation is at least partly due to patterns of colonization by other plant species; for example, one *S. latifolia* and one hybrid population in open sites became overrun with *Vicia cracca*, and both went extinct. A broad range of grasses and herbaceous perennials became established in the experimental plots in open areas.

Habitat effects on reproduction of *S. dioica*, *S. latifolia* and hybrids

The number of flowers per plant different significantly between species ($F_{2,443} = 7.71$, $P = 0.001$), being highest in *S. dioica* and lowest in *S. latifolia*, with hybrids intermediate (Fig. 2). Posthoc tests reveal that the difference between *S. dioica* and *S. latifolia* was significant ($P = 0.004$), but that the hybrids did not differ significantly from either of the

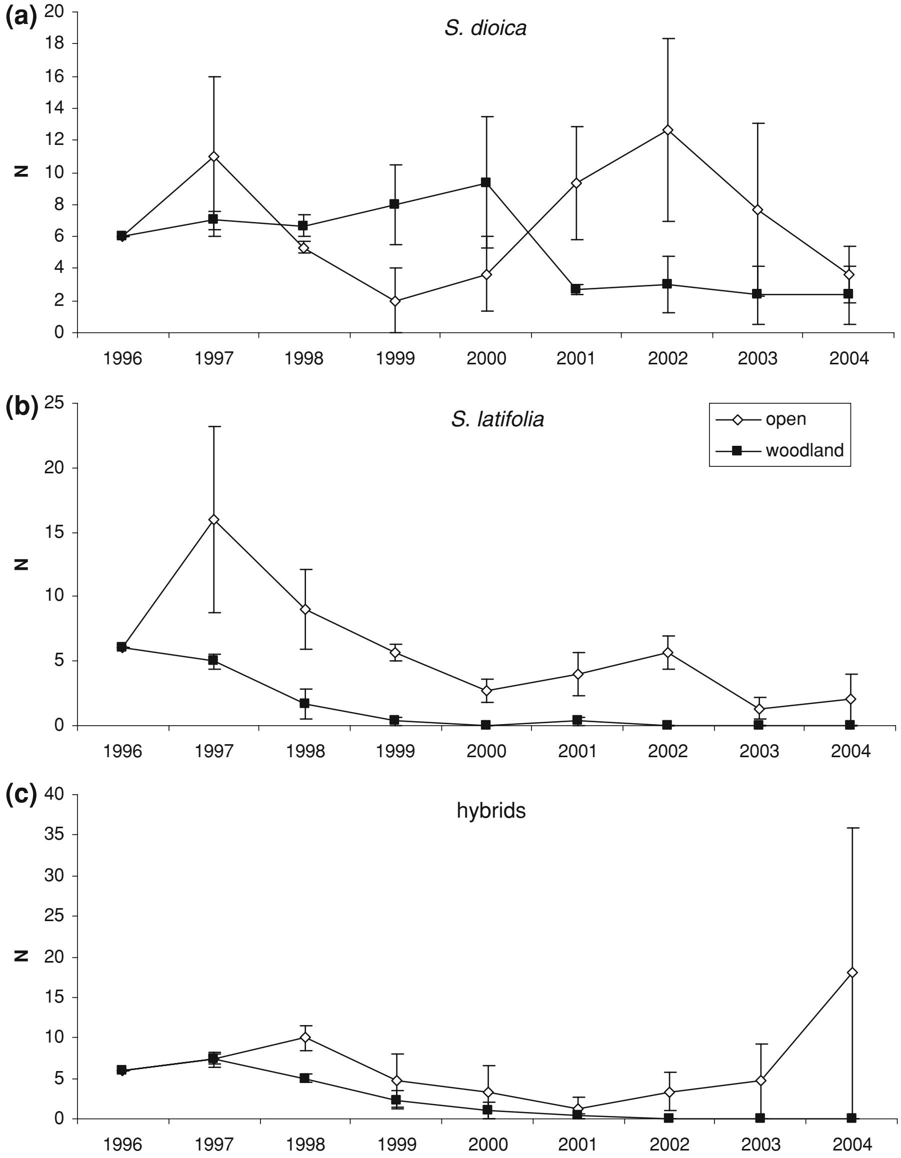

Fig. 1 Numbers of plants (all species) in 18 experimental populations founded in 1996 with 6 individuals. Each population was founded as either: **a** pure *S. dioica*; **b** pure *S. latifolia*; **c** F1 hybrids. Figures are mean numbers of plants per population (±SE). Note that the small number of plants surviving in *S. latifolia* plots at the end of the experiment were all hybrids

parental type. Males produced more flowers than females in both species and hybrids ($F_{1,443} = 4.44$, $P = 0.036$). Open versus woodland sites differed markedly; in woodland, there were very few flowers per plant of either species or hybrids ($F_{1,443} = 34.7$, $P < 0.001$), which presumably at least in part explains the low recruitment in woodland. There were no significant interactions between factors. It must be noted that these data should be interpreted with caution, since flowering was censused only once each year (chosen to approximately match the peak of flowering); if species differ in flowering phenology, then relative flower abundance may be misrepresented.

Cultivation of the open sites in the winter of 2004 did not have any marked impact on population trends, although one hybrid population did rise dramatically in number between the 2003 and 2004 census.

Table 1 Output of repeated measures ANOVA of population sizes over time, in populations founded in 1996 as either pure *S. dioica*, *S. latifolia* or entirely of hybrids

Source	Type III sum of squares	d.f.	Mean square	F	P
Between-subjects effects					
Intercept	877.340	1	877.340	34.364	0.000
Species	162.309	2	81.154	3.179	0.078
Habitat	20.056	1	20.056	0.786	0.393
Species * habitat	7.148	2	3.574	0.140	0.871
Error	306.370	12	25.531		
Within-subjects effects					
Year	470.272	5.425	86.690	12.314	0.000
Year * species	111.914	10.850	10.315	1.465	0.167
Year * habitat	15.556	5.425	2.868	0.407	0.856
Year * species * habitat	185.741	10.850	17.120	2.432	0.014
Error (year)	458.296	65.097	7.040		

Degrees of freedom have been adjusted for sphericity

In woodland populations, there was very little evidence of introgression (Fig. 3), but this is not surprising since there was very little sexual reproduction. In contrast, in open sites, there was strong and progressive introgression regardless of the starting population, and therefore, all populations came to be dominated exclusively or very largely by hybrids. This difference between habitats was significant ($F_{1,8} = 27.8$, $P = 0.001$). There was also a difference between starting species with regard to the proportion of hybrids that developed; plots founded with *S. latifolia* subsequently contained a higher proportion of hybrids than those founded as *S. dioica* ($F_{1,8} = 7.13$, $P = 0.028$). For populations founded as pure *S. latifolia*, no *S. latifolia* plants remained after 9 years.

Overall, there was a significant tendency for hybrid plants to have a female biased sex ratio (1 ♂: 1.33♀), while sex ratios in *S. dioica* (1 ♂: 0.76♀) and *S. latifolia* (1 ♂: 0.88♀) were both slightly male biased (χ^2 test of association, $\chi^2 = 7.03$, d.f. = 2, $P < 0.05$).

All of the 30 larvae reared from seed capsules proved to be *Hadena rivularis* (Noctuidae). There was no seed predation in woodland sites. Predation was most frequent against *S. latifolia* and in hybrids, compared to *S. dioica* (Table 2), but this difference was not significant ($F_{2,190} = 0.097$, n.s.). Predation varied greatly between years, with over 50% of capsules being attacked in 2000, while in contrast, in 2002, not a single case of seed predation was detected ($F_{7,190} = 5.91$, $P < 0.001$) (Fig. 4). Number of seeds per capsule did not vary significantly between species ($F_{2,114} = 2.51$, n.s.). There was a large difference between the number of seeds in damaged versus undamaged capsules ($F_{1,114} = 478$, $P < 0.001$), but no significant interaction between species and the effect of seed predation ($F_{2,114} = 2.15$, n.s.) (Table 2).

There was no correlation between the incidence of seed predation and population size (all species combined, using only plants in open sites; correlation coefficient = 0.18, n.s.) or between the seed predation rate and the population change over the following year (correlation coefficient = 0.028, n.s.)

Anther-smut (*U. violacea*) was recorded in 11 plants in total, of which nine where *S. latifolia* and two were hybrids. All cases were in open sites. Although these values are too small for statistical analysis; it seems likely that smut infections are more prevalent in *S. latifolia* than in *S. dioica*.

Discussion

Studies of chloroplast DNA variation in *S. dioica* and *S. latifolia* across Europe suggest a history of hybridization and introgression between the two species over broad geographic areas, with *S. latifolia* having distinct haplotypes only in Mediterranean

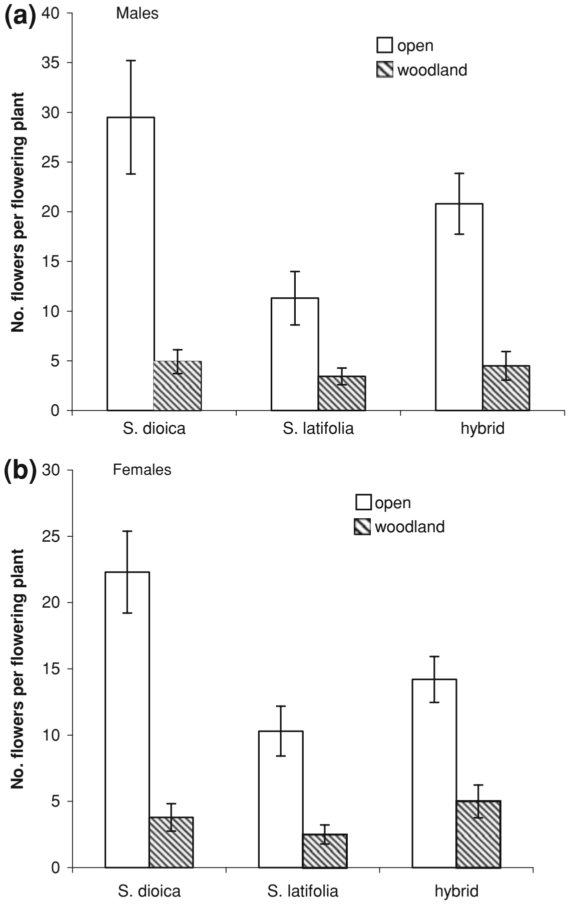

Fig. 2 Mean number of flowers per flowering plant (±SE) for *S. dioica*, *S. latifolia* and hybrids growing in open versus woodland sites, averaged over the 9 years of the experiment. **a** males; **b** females

regions where *S. dioica* does not occur (Prentice et al. 2008). There is only weak floral isolation between *S. dioica* and *S. latifolia*, and it has been suggested that species integrity must be maintained through ecological isolation, with each species being adapted to different habitats (Goulson and Jerrim 1997). The present study partially confirms this hypothesis. *S. latifolia* and hybrid plants seem unable to persist in woodland, and in nature, they are not found in heavily shaded sites (Baker 1947; Goulson and Jerrim 1997). In contrast, *S. dioica* are naturally found in woodland and woodland edge, and experimental populations placed in woodland fared better than those of *S. latifolia* and hybrids (although it must be noted that one population of *S. dioica* did go extinct, and in all experimental populations, reproduction was limited). It is thus clear how populations of *S. dioica* are protected from introgression; if pollen from *S. latifolia* does reach female *S. dioica* in woodland populations, the resulting hybrid offspring are unlikely to survive for long, and because they produce very few flowers in this habitat, they are unlikely to produce further hybrid offspring. There are some similarities here with studies of rare endemic Mediterranean plants and their more widespread congeners, which demonstrate that the rare species tend to survive in more specialised environments in which their more common relatives are unable to persist (Debussche and Thompson 2003; Lavergne et al. 2004).

It remains unclear how pure populations of *S. latifolia* persist. In open sites, there were no clear differences in the survival of *S. dioica* and *S. latifolia*, with populations of both species degenerating into hybrid swarms. The use of morphological rather than molecular markers to identify hybrids is likely to have *underestimated* the number of hybrids in the experimental populations (Minder et al. 2007; Karrenberg and Favre 2008). Hybrids suffered no obvious major disadvantage. Flower production was intermediate between parental species, and seed production per capsule has previously been shown to be higher in hybrids than in either parent (Goulson and Jerrim 1997). Recruitment of hybrids was considerable, suggesting that they have no major handicap in terms of germination or seedling predation. Seed predation did not differ significantly between species (it did differ markedly between habitats, but this should not act as a barrier to introgression). Smut infections were rare and primarily in *S. latifolia*.

A second conundrum raised by the findings of this study is why *S. dioica* is not more common in open situations, for it appeared to persist as well as *S. latifolia*, at least for the duration of this experiment and under the particular conditions at these experimental sites. It is normally found in damp and shady areas, and becomes more common in open situations in the west of Britain where rainfall is higher. It may be that it is more susceptible than *S. latifolia* to drought in particularly hot and dry years. Clearly, further studies are needed to examine this.

Hybrids did exhibit a significant female-biased sex-ratio. Similar sex-ratio distortion in hybrid *Silene* has been described before; in laboratory studies, Taylor (1994) found that hybrids between *S. dioica* and *S. latifolia* had a female-biased sex-ratio. Producing

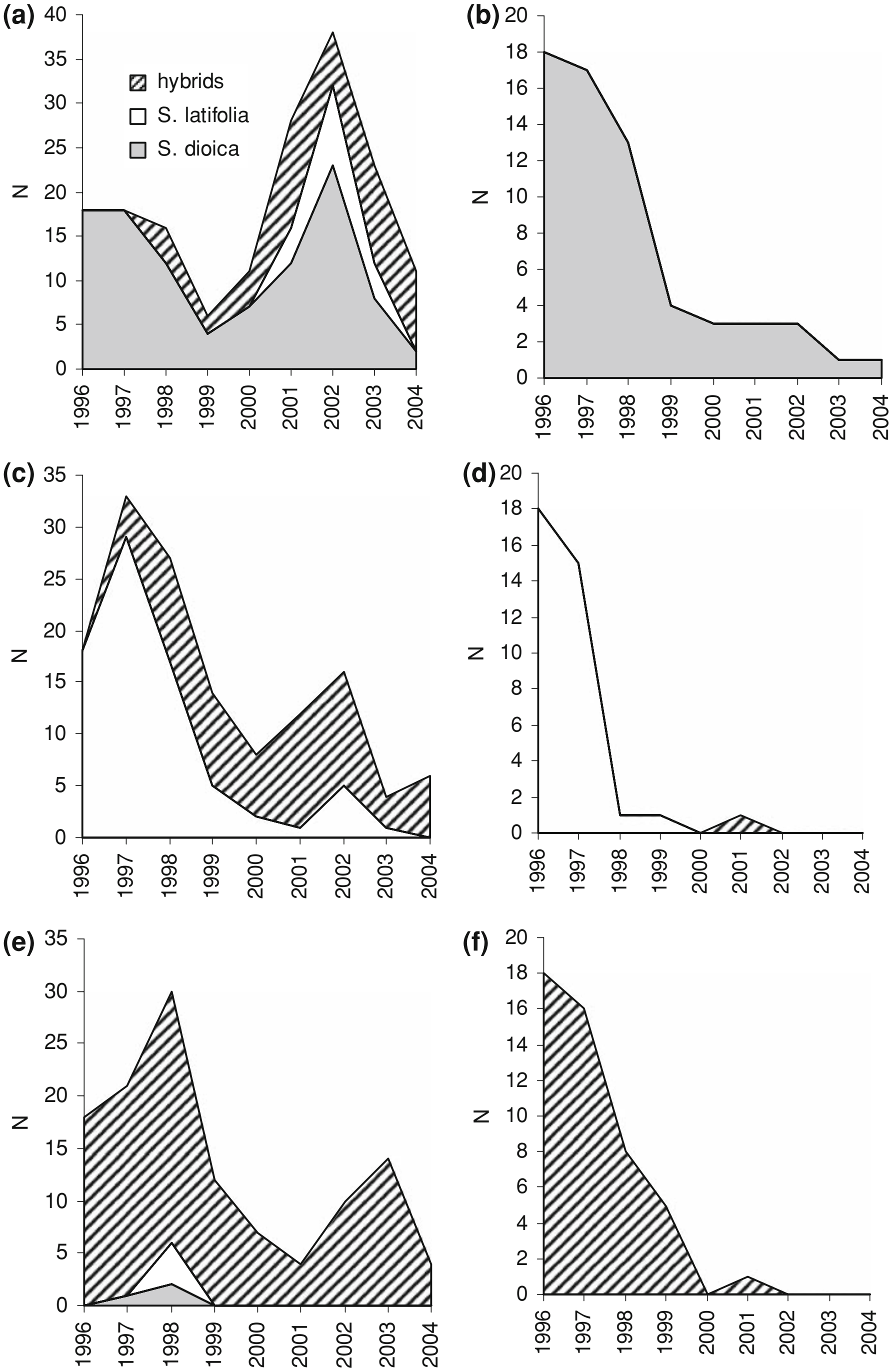

Fig. 3 Numbers of *S. dioica*, *S. latifolia* and hybrids over time, in populations founded as either pure *S. dioica*, *S. latifolia* or F1 hybrids, planted in either open sites or in woodland. Numbers are totals for each of three replicate experimental populations **a** *S. dioica*, open; **b** *S. dioica*, woodland; **c** *S. latifolia*, open; **d** *S. latifolia*, woodland; **e** hybrids, open; **f** hybrids, woodland. Only flowering plants are included as only these could be identified

female-biased offspring sex-ratios may be maladaptive at the individual level (in a female-biased population males will have higher reproductive success). However, it is unlikely to prove to be a major disadvantage at the population level. Quite the reverse, the greater number of females will result in more seed set and more recruitment, all else being equal (and assuming, as is certainly the case here, that there are sufficient males to fertilize the females). Overall, in the conditions present in these experimental sites, it is hard to envisage how pure *S. latifolia* could survive in the long term.

Table 2 Mean numbers of seeds per undamaged capsule, percentage of capsules damaged by lepidopteran predators and mean number of seeds remaining in damaged capsules for *S. latifolia*, *S. dioica* and hybrids

Species	Seeds per undamaged capsule (±SE)	% Capsules damaged (±SE)	Seeds per damaged capsule (±SE)
S. latifolia	111.9 ± 3.88	25.5 ± 6.1	24.2 ± 6.95
Hybrids	134.3 ± 2.41	24.4 ± 3.1	29.4 ± 6.56
S. dioica	129.3 ± 4.11	14.9 ± 3.9	28.4 ± 7.61

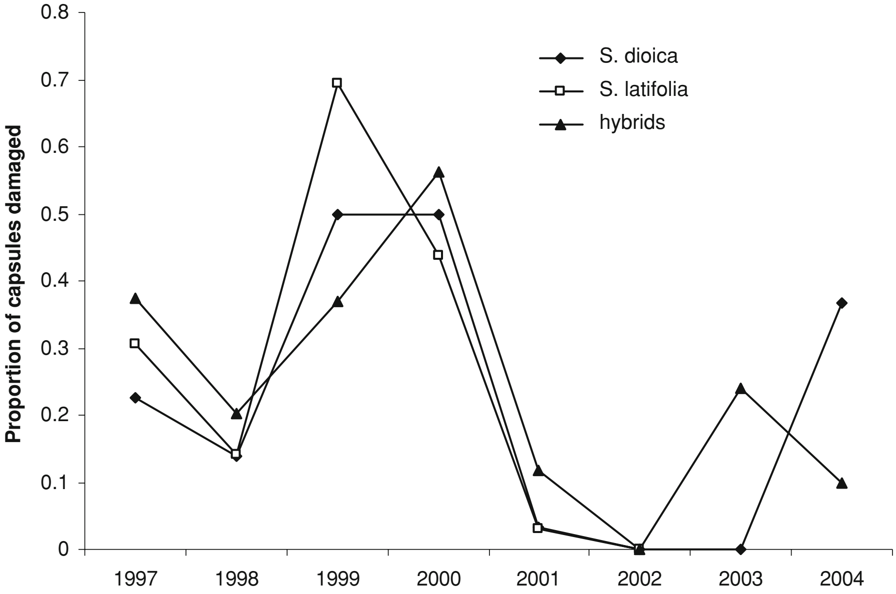

Fig. 4 Proportions of capsules damaged by seed predators (mainly or entirely *Hadena rivularis*) over the duration of the experiment. Error bars are omitted for clarity. There were no significant differences between species

One factor likely to affect population dynamics is disturbance. *S. latifolia* is a minor agricultural weed typically found in disturbed areas, while *S. dioica* is a perennial typically found in relatively undisturbed semi-natural environments (Baker 1947). Thus, one might expect disturbance to differentially affect the two species, while the response of hybrids is hard to predict. The experimental situation here provided an initially disturbed site (the plots were cultivated prior to planting), but there was no subsequent disturbance for 7½ years, when the plots were ploughed and harrowed (as might be the case in long-term set-aside). Overall, *Silene* populations in the open site tended to initially increase but then declined (although there was considerable variation), with some becoming extinct after 6–7 years. This is what one might expect for *S. latifolia* which may require disturbance for survival. However, there was no obvious difference in the response of *S. latifolia, S. dioica* and hybrids to disturbance. Only one population substantially increased following disturbance, and that consisted of hybrids. There appears to be no evidence that *S. latifolia* differentially benefits from disturbance. It should be noted that, in this study, only one disturbance event, of a particular type (ploughing), was included. Agricultural practices commonly include other forms of disturbance such as cutting and applications of herbicides which may have quite different effects on the dynamics of these species.

To summarise, this study shows that ecological isolation (niche differentiation) can maintain species integrity (of *S. dioica*) despite weak floral isolation, sympatry and fully viable hybrids. Conversely, in the absence of any known fitness costs to hybrids in open sites, the mechanism(s) maintaining integrity of *S. latifolia* remain unknown. Minder and Widmer (2008) provide evidence that species integrity is maintained by selection pressures that act upon a small proportion of the genome, but our study failed to find any evidence for differential selection in open habitats. It is possible that *S. latifolia* only persists in southern UK due to ecological isolation at a larger scale than was examined here. *S. dioica* is much more common in the wetter west of the UK, and is scarce or absent from much of the drier, agricultural landscape of eastern England and from the chalk hills of the North and South Downs to the north and east of the study area (Baker 1947, 1948a; Willmot and Moore 1973; Prentice 1979). It may be that *S. latifolia* persists in the study area through immigration from calcareous refugia. Large-scale population genetic studies of patterns of introgression would be most informative in this respect.

In models of ecological speciation, reproductive isolation evolves as a consequence of divergent natural selection, which implies reduced fitness of hybrid or intermediate phenotypes (Schluter 2000). This does not seem to be the case here. Floral isolation would also appear to be too weak to have acted as a mechanism for speciation. It seems more likely that speciation in these two species was allopatric, with divergent selection for different habitats, and with hybrid swarms only forming on secondary contact following speciation, and perhaps in part the result of human activity. It seems likely that farming practices bring *S. latifolia* and *S. dioica* into more frequent contact than would naturally occur. The typical British landscape of hedgerows (effectively, miniature woodlands that support *S. dioica*) between regularly disturbed fields, the margins of which support *S. latifolia*, provides a very large contact area between the two species. Karrenberg and Favre (2008) also report that contact zones between the two species in the Swiss Alps occur in areas of heavy man-made disturbance. Similarly, in Germany, the artificial creation of drainage ditches has been implicated in promoting introgression between *Rorippa amphibia* and *R. palustris* (Bleeker and Hurka 2001). Hence, it may be that the frequent occurrence of hybrid swarms between these two *Silene* species is, in evolutionary terms, a recent phenomenon.

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