Survival and flowering of hybrids between cultivated and wild carrots (*Daucus carota*) in Danish grasslands

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Many crop species are able to hybridize with related weedy or wild relatives, which could lead to transfer of cultivar genes, and among them transgenes, into wild populations. It is not clear, however, whether the hybrids and their descendants are able to survive and reproduce in natural habitats, as inherited cultivar traits may be maladaptive under such conditions. To test this, we produced hybrid (F_1) seeds by controlled crosses between wild (♀) and cultivated carrots (*Daucus carota* ssp. *carota* and ssp. *sativa*, respectively) and sowed them into three Danish grasslands of different age, in parallel with seeds of wild carrots. Replicate plots were sown in fall and spring. Survival and flowering of the emerging plants were monitored for the following three years. Both hybrid and wild carrots survived and flowered in highest frequency at a recently disturbed site, and much less at two older sites. Hybrids emerged in higher proportions than wild carrots in the first year and survived to similar or slightly lower frequencies at the end of the experiment. Hybrids flowered as frequently or slightly less frequently than wild plants, and developed fewer and smaller umbels. Despite a somewhat lower reproductive potential compared to wild carrots, first generation hybrids between cultivated and wild carrots are likely to survive and produce offspring in natural grasslands in Denmark. This, together with other studies, suggests that cultivar genes may transfer relatively easily into wild carrot populations.

Keywords: hybridization / crop / wild / emergence / survival / flowering

INTRODUCTION

After crops were domesticated not long ago, they have become adapted to the highly managed and nutrient-rich conditions in modern agricultural fields. Their wild relatives, in contrast, are still exposed to more stressful natural conditions, such as higher competition with other plants, attack by pests, etc. Despite these differences, most crops are still able to hybridize with wild relatives and produce viable offspring (Ellstrand, 2003). The hybrids may, however, be less fit than either parent in fields, natural habitats or both, as they inherit a mixture of adaptations to the two different environments. Small (1984) thus suggested that "domesticated features [in hybrids between cultivated and wild carrots] are simply too inadaptive to wild existence for transferred genes to survive. Natural selection in cultivation seems to effectively block gene exchange". He based his conclusion on the

lack of cultivar morphological traits in weedy carrots in North America.

Other traits from crop plants, such as efficient utilization of high nutrients levels, high growth rate, increased allocation to reproduction, and pest resistance (Spencer and Snow, 2001), may, however, benefit hybrids. Hybrids may also initially be positively affected by heterosis, especially if the wild relatives grow in small, isolated populations that are affected by inbreeding depression and deficient genetic variation (*e.g.* Hauser et al., 1998; 2004). Novel combinations of parental traits may occasionally allow hybrids to establish in habitats different from their parents (Arnold et al., 1999; Lexer et al., 2003). It is thus not clear whether hybrids between cultivated and wild plants should be expected to be vigorous or weak, especially when growing in unmanaged, natural habitats. Until now, only relatively few studies have been published on the survival and reproduction of crop-wild hybrids in natural habitats (review in Ellstrand, 2003; Hooftman et al., 2005; Kiær et al., 2007; Sørensen et al., 2007), despite its relevance for understanding ecological and evolutionary issues such as conservation of crop relatives, introgression of transgenes into

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wild populations and associated effects, and evolution of weedy and invasive ecotypes (Ellstrand, 2003; Rhymer and Simberloff, 1996). If hybrids have decreased fitness in natural habitats, transfer of crop genes into wild populations, and thus evolution of invasive and weedy types (due to uptake of cultivar traits) should be infrequent. In contrast, if hybrids are doing fine, then genetic introgression and evolution of invasive and weedy types would be more likely.

Cultivated and wild carrots (*Daucus carota* L. ssp. *sativa* (Hoffm.) Arc. and ssp. *carota*, respectively) hybridize easily if hand-pollinated (Dale, 1974; Heywood, 1983). Spontaneous hybridization can take place in seed production fields where surrounding wild carrots pollinate seed plants (references in D'Antuono, 1985; Heywood, 1983). The resulting hybrid seeds are then transported intermixed with commercial seeds to other cultivation regions, *e.g.* Denmark. Here, the hybrids sometimes flower and pollinate each other and probably neighboring wild plants (Hauser and Bjørn, 2001). Pollen may also spread from cultivar seed production fields into neighboring wild populations (Wijnheijmer et al., 1989). Finally, pollen may flow from flowering (bolting) cultivar individuals in root production fields into adjacent wild carrot populations (Magnussen and Hauser, 2007). Similar hybridization routes have been described for cultivated beets (*Beta vulgaris*) (Arnaud et al., 2003; van Dijk, 2004). Which hybridization route is most frequent in carrots depends on the cleanliness of the seed production and weeding intensity in and adjacent to the carrot fields (Hauser and Bjørn, 2001).

The potential for spontaneous hybridization is relatively high, as wild carrots grow in ruderal and open habitats in many parts of the world, often introduced as seed impurities (Dale, 1974), and at least in Denmark often close to carrot fields. Cultivated carrots have been bred to become biennial, to avoid flowering in the root crops; however, some cultivar plants often flower after a cold spring (Atherton et al., 1990). Wild carrots are annual, biennial or perennial, though all types reproduce only once before dying (Dale, 1974; Gross, 1981). Wild and cultivated carrots seem to flower in synchrony, at least under Danish conditions (T.P. Hauser, pers. obs.). In both carrot types, flowering within umbels is usually synchronized with a clear separation between male and female phase (Koul et al., 1989; T.P. Hauser, pers. obs.); carrots are thus usually outcrossing, albeit able to self-fertilize (Thompson, 1962).

Even if carrot F_1 hybrids are produced spontaneously, Small's (1984) argument that cultivated traits are "inadaptive to wild existence", may still be correct. *E.g.*, hybrids between cultivated and wild carrots are less frost tolerant than wild plants (Hauser, 2002), and formation of later generation hybrids and introgression of genes into

wild populations could therefore be strongly reduced in frost-prone habitats.

To test whether hybrids from pollinations of wild carrots by cultivar pollen are able to survive and reproduce in natural, unmanaged, habitats, we sowed seeds from controlled hybridizations, and seeds from wild carrots, into three Danish grasslands of different successional age. All sites contained viable populations of wild carrots already, indicating that their conditions were suitable for wild carrots. During the following three years, we monitored emergence, survival, and flowering of the sown carrots, and analyzed the following questions: (1) Do the F_1 hybrids survive to flowering in the three different habitats? (2) Do they flower? (3) Are they much less likely to survive and flower than the wild carrots also included in the experiment?

RESULTS

Emergence and survival

The number of plants that emerged from seeds differed strongly among sites in the first year (Fig. 1). At Amager, a recently disturbed site, approximately half the seeds germinated and survived to their first summer, whereas < 20% germinated and survived at Hedeland, an older site on poor soil. Some additional plants emerged after germination in the second (2000) and third (2001) years, especially in Hedeland (Fig. 1b). The number of plants at Amager continued to be highest until the end of the experiment (Fig. 1a). Out of 36 000 seeds sown, 1066 individuals survived to the end of the experiment.

Plants emerged in higher frequencies from the mixed seed lots (containing approx. 68% F_1 hybrids and 32% wild seeds; see Materials and Methods) than from the wild lots (containing only wild seeds) at Amager and Risø in the first year (Figs. 1a and 1c). The estimated survival rates for F_1 hybrids (s_{01}^h) were therefore significantly higher than for wild plants (s_{01}^w; Tab. 1, Figs. 2a and 2c). Hybrids survived in higher frequencies than wild plants until 2000 ($t = 2$; Tab. 1, Figs. 2a and 2c). This changed, and hybrids survived less frequently than wild plants to the end of the experiment at Amager and in the autumn-sown plot at Risø, but slightly more frequently in the spring-sown plot at Risø (Figs. 2a and 2c). However, at that time only few plants were left (Figs. 1, 2a and 2c). At Hedeland, hybrids emerged in higher frequencies than wild plants in the spring-sown plot in the first year, but in lower frequencies in the autumn-sown plot; there was no difference in hybrid and wild survival to the end of the experiment (Tab. 1, Fig. 2b). The possibility that all hybrids died and only wild carrots were left in the mixed rows was highly unlikely at all censuses (test of model with F_1
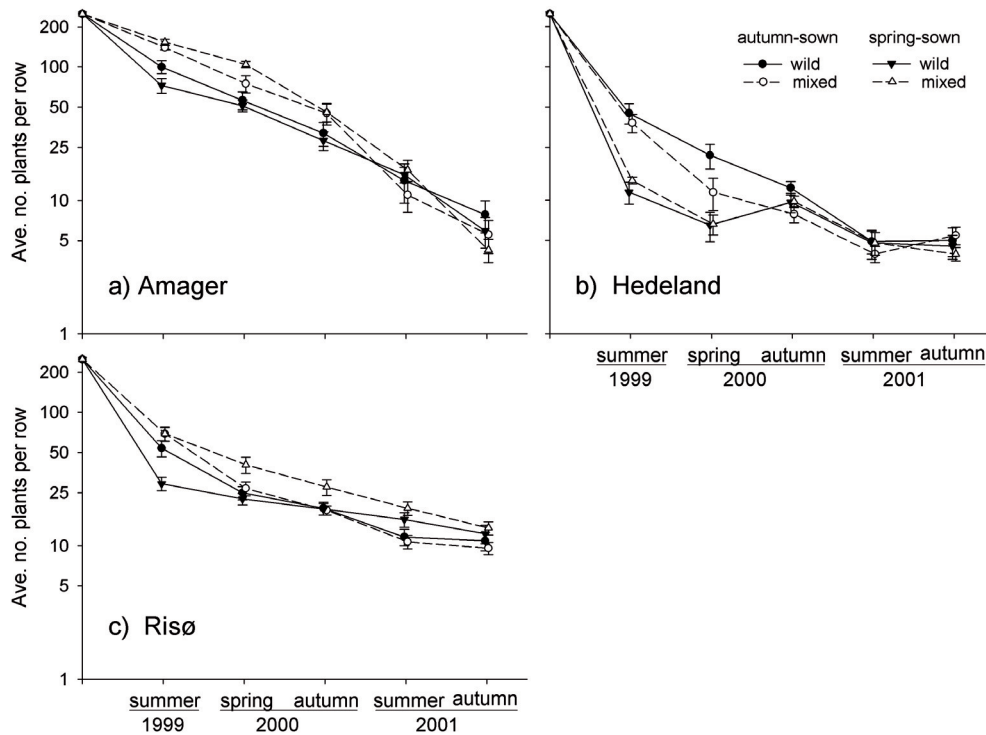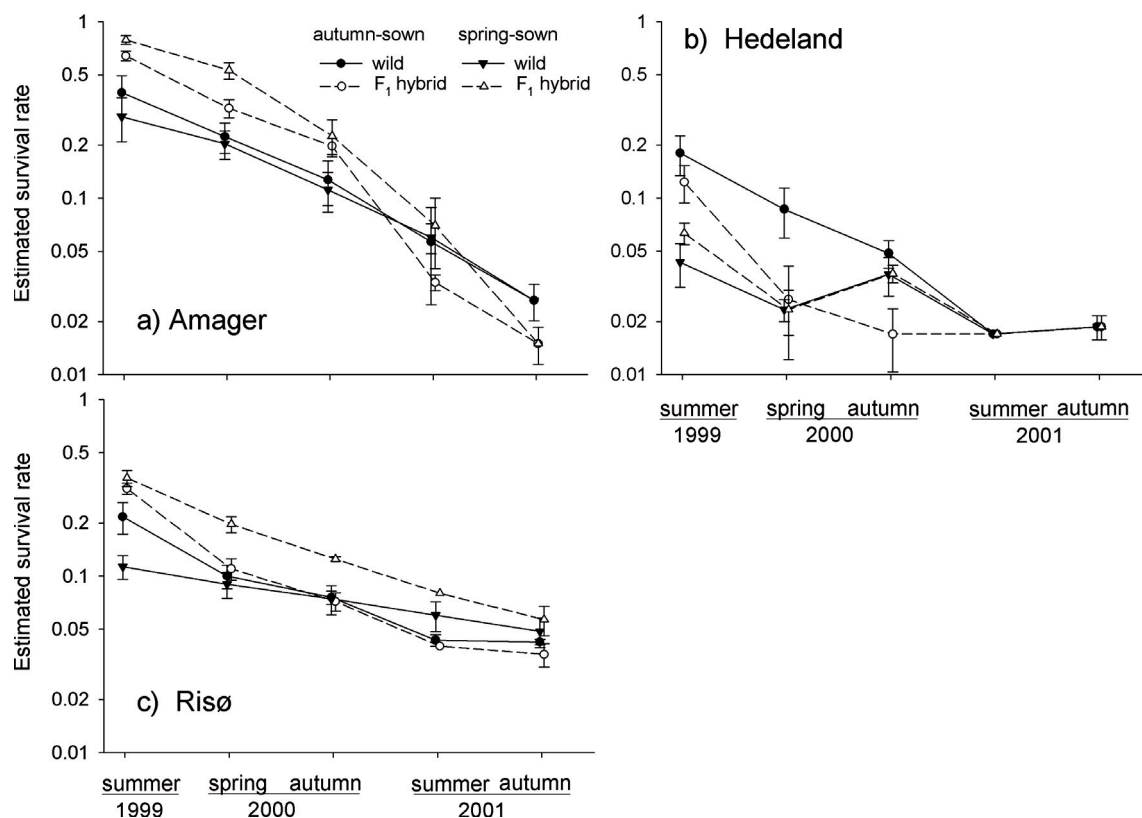
Figure 1. Observed number of carrot plants in wild and mixed rows during the three years of the experiment, shown for autumn and spring-sown plots at a) Amager, b) Hedeland, and c) Risø. Two hundred and fifty seeds were sown into each row in the autumn 1998 or early spring 1999. Error bars indicate standard errors among rows; notice log-scale.

Table 1. Test of hypotheses on survival and flowering proportions: H_{pop}: Are maternal populations similar? ($s_{0t\,j1}^{i} = s_{0t\,j2}^{i} = s_{0t\,j3}^{i}$), H_{type}: Are wild and hybrid plants similar? ($s_{0t\,jk}^{w} = s_{0t\,jk}^{h}$), and H_{plot}: Are autumn and spring-sown plants similar? ($s_{0t\,1k}^{i} = s_{0t\,2k}^{i}$). Log-likelihoods are given for the full model (including estimates for each maternal population, plant type, and plot) and for constrained models of the three hypotheses, with associated chi-square probabilities of the deviances. See Materials and Methods for more detail. ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$; ns: $p > 0.05$.

	H	**Amager** ln(L)		**Hedeland** ln(L)		**Risø** ln(L)			**H**	**Amager** ln(L)		**Hedeland** ln(L)		**Risø** ln(L)	
s_{01}	Pop	−462	***	−312	***	−401	***	s_{04}	Pop	−281	***	−116	ns	−165	***
	Type	−921	***	−288	***	−567	***		Type	−242	***	−117	ns	−164	***
	Plot	−292	***	−423	***	−417	***		Plot	−252	***	−113	ns	−178	***
	Full	−208		−243		−346			Full	−206		−111		−156	
s_{02}	Pop	−461	***	−243	***	−234	***	fl_{02}	Pop	−178	***	−82	***	−69	*
	Type	−670	***	−253	***	−271	***		Type	−165	***	−25	ns	−69	**
	Plot	−445	***	−260	***	−243	***		Plot	−178	***	−24	ns	−67	**
	Full	−373		−197		−212			Full	−148		−18		−59	
fl_{01}	Pop	−368	***					s_{0end}	Pop	−165	***	−126	*	−162	*
	Type	−393	***						Type	−165	***	−0.02	ns	−163	**
	Plot	−368	***						Plot	−153	ns	−120	ns	−165	***
	Full	−305							Full	−150		−117		−153	
s_{03}	Pop	−476	***	−137	***	−184	**								
	Type	−529	***	−139	**	−193	***								
	Plot	−400	***	−131	***	−190	***								
	Full	−385		−197		−172									

Figure 2. Estimated survival rate for wild ($s^w_{0t\,j}$) and F_1 hybrid ($s^h_{0t\,j}$) carrots from the start of the experiment to the different census times, shown for autumn and spring-sown plots at a) Amager, b) Hedeland, and c) Risø. Symbols and error bars indicate averages and standard errors among maternal seed populations, respectively; notice log-scale. See Materials and Methods for more detail.

survival constrained to zero, see Materials and Methods; $p < 0.0001$ in all tests).

Many more plants were observed in the autumn-sown plot than in the spring-sown plot at Hedeland in the first summer and second spring (Tab. 1, Fig. 1b), and estimated survival was therefore higher (Fig. 2b). Maternal populations differed in the emergence and survival of their offspring (Tab. 1), but were affected in similar ways by plant type (hybrid or wild) and sowing time.
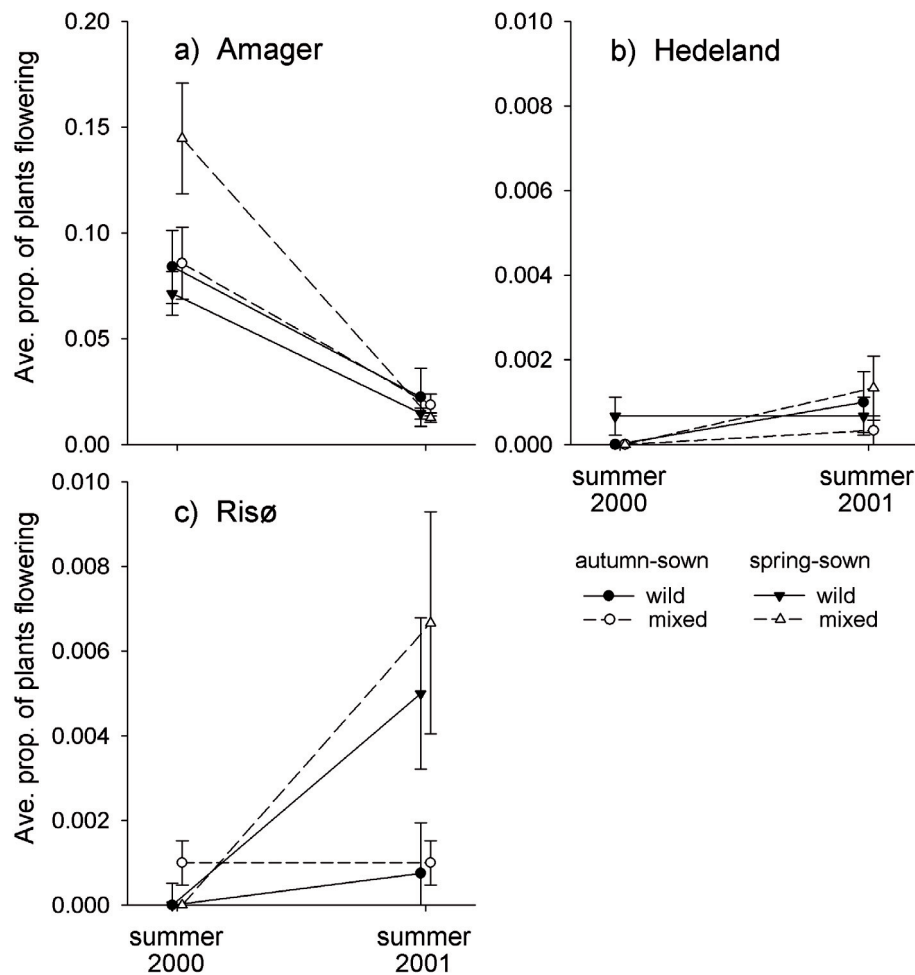
Flowering

No plants flowered in the first summer (1999), many plants flowered at Amager in the second summer (2000), and few plants flowered at Amager and Risø in the third summer (2001) (Figs. 3a and 3c); very few plants flowered at Hedeland at all (Fig. 3b). There was no difference in flowering time between plants in wild and mixed rows (T.P. Hauser, pers. obs.).

In the spring-sown plots at Amager, higher proportions of plants flowered in the mixed rows than in the wild (relative to the number of seeds sown) in the sec-

ond summer (2000, Fig. 3a). There was no clear difference between mixed and wild rows in the autumn-sown plots. The estimated flowering probabilities were thus higher for F_1 hybrids (fl^h_{02} and fl^h_{03}) than for wild plants in spring-sown plots, but lower in autumn-sown in both years (average across maternal populations; Tab. 1, Fig. 4a). At Risø, plants in the mixed rows flowered on average in slightly higher proportions than those in wild rows (Fig. 2c), however, these proportions were very low. The estimated flowering probabilities for F_1 hybrids, fl^h_{02} and fl^h_{03}, were slightly higher for F_1 hybrids than for wild plants (Tab. 1, Fig. 4c). At Hedeland, the proportions of flowering did not differ between wild and F_1 plants. It was highly unlikely ($X^2 = 1708$, df $= 1$, $p < 0.0001$) that only wild plants, and not F_1 hybrids, flowered in the mixed rows.

Only flowering plants at Amager were analyzed for height, number of umbels, and umbel size, as there were too few flowering individuals at the other sites for analysis. Flowering individuals in the mixed rows were taller than those in wild rows (results not shown), but set significantly fewer umbels (ca. one less; ANOVA:

Figure 3. Proportions of sown seeds that give rise to flowering plants in 2000 and 2001 (second and third year after sowing), shown for wild and mixed rows in autumn and spring-sown plots at a) Amager, b) Hedeland, and c) Risø. Error bars indicate standard errors among rows; notice different scales in the panels.

F = 6.76, df = 1, p = 0.01) that were significantly smaller (ANOVA: F = 5.95, df = 1, p = 0.02; Tab. 2). The estimated number and size of umbels on F_1 hybrids was accordingly even smaller (Tab. 2). All umbels carried healthy-looking green developing seeds when harvested.

Maternal populations differed in the proportion of flowering and size of their umbels; however, these were affected in similar ways by plant type (hybrid or wild) and sowing time.

DISCUSSION

Our study indicates that F_1 hybrids between wild (♀) and cultivated (♂) carrots are able to survive and flower in natural habitats. They emerged from seeds and survived to the end of the experiment three years later in almost as high frequencies as wild carrots. They flowered as frequently as or slightly less frequently than wild

carrots, and produced somewhat fewer and smaller umbels, indicating that they may have a somewhat lower seed production that wild plants (but see discussion below). Our results are thus in agreement with a parallel study that found advanced-generation hybrid adult plants (F_2, B_1 or later generations) growing in wild populations close to a major carrot production region in Denmark (Magnussen and Hauser, 2007), indicating that F_1 hybrids in an earlier generation survived and reproduced. The same study also showed that wild populations close to carrot fields are genetically more similar to cultivated carrots than wild populations far from carrot fields, indicating that hybridization and introgression of cultivar genes into surrounding populations may be frequent (Magnussen and Hauser, 2007). Our results are also in agreement with results of Wijnheijmer et al. (1989), who found carrots with a morphology intermediate to wild and cultivar plants growing close to a Dutch carrot seed fields.
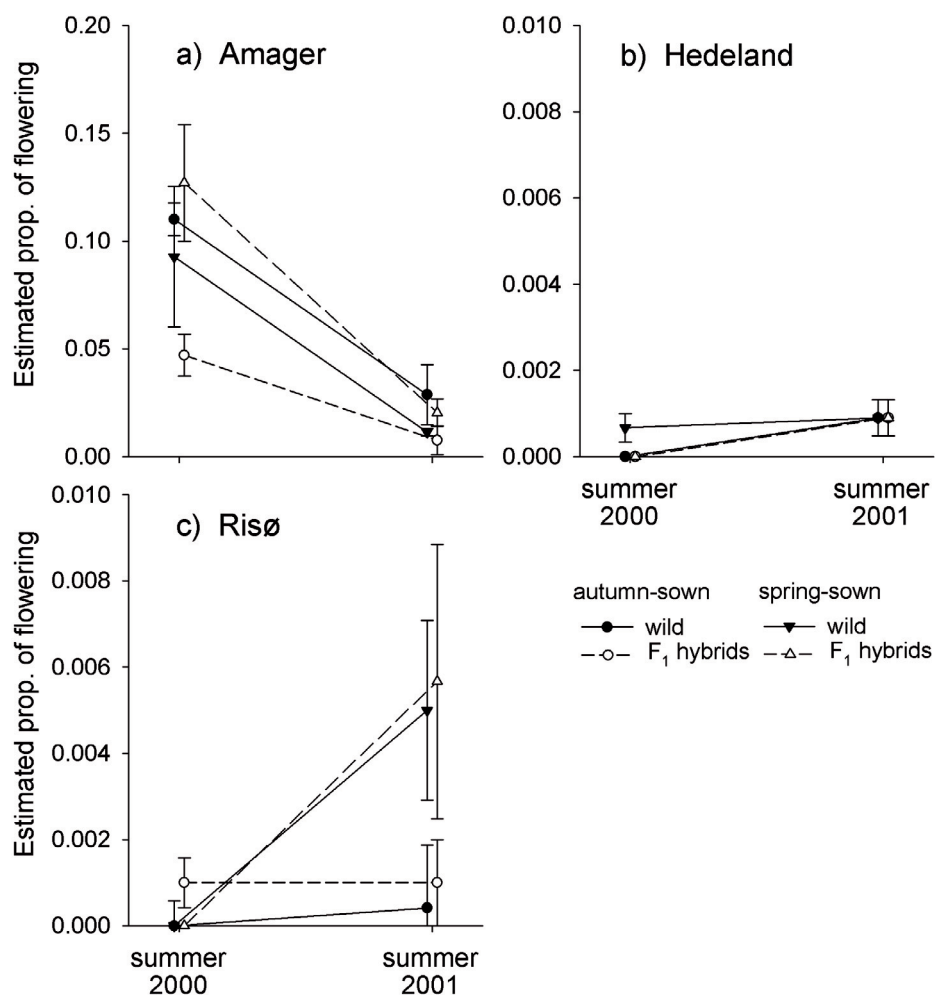
Figure 4. Estimated proportions of wild ($fl_{0t\ j}^{w}$) and F$_1$ hybrid ($fl_{0t\ j}^{h}$) seeds that give rise to flowering plants in 2000 and 2001 (second and third year after sowing), in autumn and spring-sown plots at a) Amager, b) Hedeland, and c) Risø. Symbols and error bars indicate averages and standard errors among maternal seed populations, respectively; notice different scales in the panels.

Table 2. Number of umbels per plant and their radii (cm) at Amager summer 2001, shown for each maternal population and for wild (*w*) and mixed rows (*m*). In addition is given an estimate for pure F$_1$ hybrids in the mixed rows (*h*). Standard error and sample sizes in parentheses.

	HUN			KAR			KIK		
	wild	*mixed*	*hybrid*	*wild*	*mixed*	*hybrid*	*wild*	*mixed*	*hybrid*
Umbels per plant	4.2 (0.5, 21)	3.6 (0.4, 24)	1.6	3.2 (0.3, 22)	2.4 (0.3, 24)	2.2	3.1 (0.2, 23)	2.3 (0.4, 23)	1.9
Radius umbel 1	4.19 (0.17, 21)	3.63 (0.20, 24)	2.8	4.30 (0.19, 22)	3.18 (0.16, 24)	2.9	3.17 (0.14, 23)	3.33 (0.17, 23)	3.4
Radius umbel 2–4	2.30 (0.14, 21)	2.03 (0.13, 21)	1.5	2.47 (0.18, 21)	2.08 (0.20, 16)	1.9	2.01 (0.16, 21)	1.90 (0.19, 12)	1.8

Our results could be biased by inbreeding depression affecting wild seeds in the mixed seed lots. Due to the small flower size in carrots, large number of flowers, and only two potential seeds per flower, it was not feasible to emasculate flowers before the controlled hybridizations. Approximately 32% of the seeds produced therefore resulted from unintended wild × wild pollinations and probably from self-pollinations within and among umbels on maternal plants. Carrots are usually outcrossing (Thompson, 1962), and selfed seeds are therefore probably affected by inbreeding depression; no studies of this are available to our knowledge. In our analyses, we assumed that survival and flowering of wild plants in a mixed row was similar to their survival and flowering in the neighboring wild row. If wild plants in mixed rows were less fit than wild plants in wild rows due

to inbreeding depresssion, this difference would lead to overestimation of F_1 survival and flowering in our analyses. The magnitude of overestimation can be estimated by the product of inbreeding depression and frequency of contamination (as performance of F_1 hybrids in our analyses was estimated from the observed plant performance in mixed rows, corrected for the frequency of unintended wild plants times performance of wild plants in wild rows; see Materials and Methods), which is realistically about 15% (assuming 50% inbreeding depresssion in a plant with mixed mating system; Husband and Schemske 1996). However, some seeds from wild populations would probably also originate from self-pollinations and be affected by inbreeding depression, and the overestimation would therefore be even smaller. To our knowledge, no estimates of selfing rate are available for natural wild carrot populations, and we can therefore only conclude that this potential bias probably would be less than 15%. Our conclusion that hybrids are able to survive and reproduce in natural habitats would not be changed by this.

Our results could further have been biased by our estimation of hybrid frequencies in the mixed seed lots. Plants were scored as hybrids if they carried an AFLP band that was present in all cultivar parents of our crosses but not in the wild. All three parental cultivars were "open-pollinated" (produced by random mating in the previous generation; personal information, O.M. Madsen, carrot breeder, Dæhnfeldt A/S), and their genotype frequencies should therefore follow Hardy-Weinberg proportions. The band was also present in all cultivar plants (45 open-pollinated and 30 F_1 plants) tested by Shim and Jørgensen (2000). We therefore assumed that the band was homozygous in our cultivar parents. If it was, however, heterozygous in a few parents, we would have underestimated the proportions of hybrids, overestimated their initial survival rates, and underestimated their final survival rates and flowering traits. In the extreme, if all offspring in the "mixed rows" were indeed hybrids, their true survival and flowering estimates would have been as showed for mixed rows in Figures 1 and 3. Our conclusions are therefore robust to influence from this potential bias.

Hybrids emerged in higher proportions than wild plants in the first summer in Amager and Risø. This was most likely due to low seed dormancy, inherited from their cultivar parents. Wild carrots, in contrast, have pronounced seed dormancy, and seeds may remain dormant for two to five years (Clark and Wilson, 2003; Lacey, 1982). In agreement with this, more wild plants emerged from autumn-sown plots, where they were exposed to dormancy-breaking winter conditions, than from spring-sown plots. Also, extra plants were observed in especially wild rows in the second and third year (*e.g.* Fig. 1b).

Due to the new recruitment of especially wild plants, and death of especially hybrid plants, hybrid plants survived to the end of the experiment in lower proportions than wild plants at Amager and in autumn-sown plot at Risø. Some wild seeds may have remained alive and germinated after termination of the experiment; however, after three years of germination opportunities, only few, if any, seeds would be left and able to germinate in the now completely recovered and dense vegetation (Clark and Wilson, 2003; Gross and Werner, 1982).

The high survival of hybrids was unexpected, as they are less tolerant to frost than wild carrots (Hauser, 2002; tested on the same hybrid seed lots as in this experiment). Hybrids probably inherit frost sensitivity from their cultivar parent, as cultivated carrots only tolerate mild frost (Tucker, 1974; Tucker and Cox, 1978). The winters of our experiment (1999–2001) were relatively mild and may not have killed hybrids as much as in harsher winters.

Wild Danish carrots are clearly not annual, as reported for Dutch wild carrots (Wijnheijmer et al., 1989), since no carrot plants flowered in their first summer. Flowering year depends on size, which again depends on local environmental factors such as surrounding vegetation age and density (Gross, 1981; Lacey, 1988). This might also explain why fewer plants flowered at the more densely vegetated sites, as also found by Gross and Werner (1982). Wijnheijmer et al. (1989) suggested that the bienniality of cultivated carrots is inherited dominantly and that hybrids between cultivated and wild carrots thus should flower in their second year. All plants in the mixed rows of Hedeland and Risø were triennial or even longer lived, like the wild ones. In contrast, hybrid carrots (cultivated ♀ × wild ♂), which are produced spontaneously in seed production fields in *e.g.* southern Europe by pollen from wild carrots, are all annuals when growing within Danish carrot root fields (Hauser and Bjørn, 2001). This may be due to the beneficial conditions in fields (less competition, more nutrients) that allow hybrids to grow beyond a minimum size for flowering, or to inheritance of annuality from their wild southern fathers. Wild carrots from lower latitudes in North America are thus more annual than those from higher latitudes (Lacey, 1988).

Hybrids flowered on average in slightly lower proportions than wild carrots, and produced fewer and smaller umbels. The number and size of umbels is, however, not directly proportional to total seed yield. First order (top) umbels develop a higher proportion of mature seed than second order (first branch) umbels, and a much higher than third order umbels (Koul et al., 1989). All flowering plants developed healthy-looking green seeds that were well beyond the initial abortion stage when harvested. So the lower flower production of hybrids, suggested by fewer and smaller umbels, may translate into

a smaller difference in the number of mature seeds produced. In any case, F_1 hybrids will produce seeds in high (absolute) numbers; a single successful umbel of wild carrots may produce between 800 and 3500 seeds (Koul et al., 1989; Lacey, 1982). Results from Hauser and Bjørn (2001) show that cultivar (♀) × wild (♂) hybrids can produce large quantities of mature and viable seeds and offspring, and that their pollen is as viable as cultivar pollen (ca. 76%).

Our results clearly refute the argument by Small (1984) that domesticated traits, inherited from cultivated carrots, inhibit survival of carrot hybrids in wild habitats. Our results only pertain to the F_1 generation, though. Fitness of subsequent generations of hybrids will depend strongly on the crossing combinations creating these generations. The most likely offspring produced by F_1 plants in a wild carrot population will be from backcrosses to wild plants, if F_1 plants are infrequent. The fitness of successive generations of backcross descendants will gradually approach that of wild plants, but may to some extent be affected from outbreeding depression, through the breakdown of coadapted gene complexes (Lynch and Walsh, 1997). However, outbreeding depression may not be severe, as they inherit a complete (haploid) set of genes from the wild parents each generation. Advanced hybrids have thus been found in wild populations close to carrot fields (Magnussen and Hauser, 2007). Hybrids of the reciprocal crossing direction, cultivar (♀) × wild (♂), can form semi-permanent weedy populations in Danish carrot fields (Hauser and Bjørn, 2001), indicating that outbreeding depression is not severely affecting hybrid descendants of this constitution.

Other recent studies have reached similar conclusions as ours, that hybrids between highly domesticated and related weedy and wild types sometimes do surprisingly well (*Lactuca*: Hooftman et al., 2005; *Cichorium*: Kiær et al., 2007, Sørensen et al., 2007; *Helianthus*: Mercer et al., 2007; reviewed by Ellstrand, 2003), even though fitness of hybrids is strongly dependent on population composition and environmental conditions (Campbell et al., 2006; Hauser et al., 2003; Mercer et al., 2007). Movement of cultivar genes into wild populations by hybridization should perhaps be considered the norm for many species and not the exception (Ellstrand, 2003). Given the huge numbers of crop plants in fields relative to the typical numbers of wild relatives when they occur in and nearby cultivated fields, the potential for interactions *via* hybridization is large.

MATERIALS AND METHODS

Plant material

Wild carrots for this study originated from three locations in Denmark, in order to increase the genetic basis and generality of our results: Hundested (HUN): an old grassland site in Northern Zealand with many large carrot individuals; Karlstrup (KAR): an abandoned lime quarry in central Zealand with sparse vegetation containing many small carrot plants; Kikhavn (KIK): a roadside in Northern Zealand, containing few medium-sized individuals. Mature seeds were collected from each site and sown into pots in the greenhouse in autumn 1997, and after a couple of months plantlets were transferred to an outdoor bed to vernalize.

Vernalized roots of cultivated carrots were kindly provided by the seed company L. Dæhnfeldt A/S. Three open-pollinated cultivars (male fertile and produced by free pollination among parental plants) were used, Fancy (FAN; a Nantes-type cultivar), Grosa and Regol (GRO and REG; both Flakkeer-type cultivars). The roots were planted in pots in March, and kept part of the time outdoor to synchronize flowering with the wild carrots.

At flowering, wild and cultivar plants were kept in separate mesh tents (pore size 0.9 mm) to avoid unintended pollinations. A total of 43 wild carrot plants were used as pollen recipients (HUN: 15, KAR: 12, KIK: 16), and 30 cultivar plants as pollen donors (FAN: 10, GRO: 13, REG: 7). When all the stigmas on a given umbel were receptive (after all anthers had dehisced), the umbel was dusted with a male phase umbel from one of the donors. Four umbels on each recipient (top umbel and three 2nd order umbels) were pollinated with the same donor. Pollinations included all combinations of recipient populations and donor cultivars. Umbels were harvested when dry, and the seeds pooled for each maternal plant.

Since emasculation was not possible, unintended pollinations, either within or between wild maternal plants, could take place. We therefore genotyped all parental plants and a subset of their offspring (eight per maternal plant), using AFLP markers specific to the cultivar parents, to estimate the realized percentage of F_1 hybrids.

DNA was extracted from 30-day-old seedlings, and analyzed by AFLP (Amplified Fragment Length Polymorphism; methods in Shim and Jørgensen (2000)). One band, E-CAC/M-AC: 450 bp, was absent in all wild parents but present in all cultivar parents and in all plants of five cultivars surveyed by Shim and Jørgensen (2000). We therefore assumed that it was homozygous in the pollen donors, and offspring plants were determined to be hybrids if they carried this band. From this, we estimated the following frequencies of F_1 hybrids ($p_{0 \cdot k \cdot}^h$, see below): Hundested (HUN): 0.58, Karlstrup (KAR): 0.62, Kikhavn (KIK): 0.75. Differences among populations were significant.

Field experiment

Seeds from the controlled crosses (hereafter "mixed seeds") were pooled within each maternal population with an equivalent number of seeds from each maternal family. The three mixed populations thus originated from crosses with all three cultivars, in addition to possible unintended wild $\times$ wild seeds. Wild seeds originated from at least 15 plants from each of HUN, KAR, and KIK. Both wild and hybrid seeds were stored in a cold room (at 5 °C) for at least two months before sowing.

The experiment was carried out in three different unmanaged grassland sites on Zealand, Denmark: Amager: a recent construction site with very sparse vegetation the first year; Hedeland: an old gravel pit with thin and low vegetation; Risø: old clay soil fill with dense old grass vegetation. All sites contained naturally occurring wild carrots, both young and old, at the beginning and end of the experiment.

At each site, two plots were prepared, one in November 1998 (autumn plot) and one in March 1999 (spring plot). In each of the plots, 24 linear rows were cut in the turf, 2.5 m long and ca. 1 cm deep. The rows were placed in a grid of eight parallel rows in three blocks, with a distance of 1.5 m between neighboring rows. Each of the six seed populations (three mixed and three wild) was sown into four of the rows according to a Latin square design. Wild and mixed seeds from a given maternal population were always sown into neighboring parallel rows to minimize environmental differences between the two, which could otherwise lead to unreliable estimates of F_1 hybrid survival and flowering (see below). Two hundred and fifty seeds were sown into each row, *i.e.* 6000 in total for each population, and 36 000 seeds in the complete experiment.

The number of plants was counted twice each year ($t_1 = $ July 1999, $t_2 = $ May 2000, $t_3 = $ October 2000, $t_4 = $ June 2001, and $t_5 = $ September 2001). Only individuals growing within the cut rows were considered to originate from sown seeds, and each row was counted twice at each census. The number of flowering plants was counted in July 2000 and 2001. Three random individuals that had flowered were harvested from each row at Amager in September 2001, and the number of umbels and their maximum radius was measured. Developed seeds were not counted, as plants had to be removed before seed dispersal due to restrictions by the land owners. At the other two sites, the number of flowering plants was too small to analyze. The experiment was terminated in October 2001.

Data analysis

To estimate survival of F_1 plants in the mixed rows, we had to correct for the proportion of plants that did not originate from our controlled hybridizations. We therefore used a binomial model that combined the observed emergence and survival of plants in mixed rows, in neighboring wild rows and the frequency of F_1 seeds in the mixed row at the start of the experiment. Notice that we use here the term survival as the probability of producing a surviving offspring at a given time from a sown seed; this includes germination probability and ignores seed dormancy beyond the three years of the experiment, see Discussion. By assuming that the survival of wild plants in a mixed row is similar to that in the neighboring wild row, we can express the average survival of plants in the mixed row from the beginning of the experiment ($t = 0$) to time t as

$$s_{0t}^{m} = p_0^h * s_{0t}^h + (1 - p_0^h) * s_{0t}^w, \qquad (1)$$

where s_{0t}^{m} is the average survival of plants in the mixed row from the beginning of the experiment ($t = 0$) to t, s_{0t}^h the survival of F_1 hybrids in the mixed row, s_{0t}^w the survival of wild plants in the neighboring wild row, and p_0^h the percentage of F_1 hybrids in the mixed seed lots at the start of the experiment (as determined by AFLP).

In each of the three sites, the probability of observing $N_{t\,jkl}^m$ surviving plants in a mixed row at time t in plot j, population k and row l, out of $N_{0\,jkl}^m$ seeds sown, is binomially distributed with parameter $s_{0t\,jk}^m$. The probability of observing $N_{t\,jkl}^w$ plants out of $N_{0\,jkl}^w$ seeds sown in the neighboring wild row is likewise distributed with parameter $s_{0t\,jk}^w$. The logarithm of the product of the two binomial distributions gives the likelihood function

$$\ln L =$$

$$\sum_{j=1}^{2}\sum_{k=1}^{3}\sum_{l=1}^{4}\left\{ \begin{array}{l} \ln\left[\binom{N_{0\,jkl}^m}{N_{t\,jkl}^m}(s_{jk}^m)^{N_{t\,jkl}^m}(1-s_{jk}^m)^{(N_{0\,jkl}^m - N_{t\,jkl}^m)}\right] + \\ \ln\left[\binom{N_{0\,jkl}^w}{N_{t\,jkl}^w}(s_{jk}^w)^{N_{t\,jkl}^w}(1-s_{jk}^w)^{(N_{0\,jkl}^w - N_{t\,jkl}^w)}\right] \end{array} \right\}.$$
$$(2)$$

The unknown parameters $s_{0t\,jk}^m$ and $s_{0t\,jk}^w$ were estimated by maximum likelihood estimation of (2), using the Solver add-in of Microsoft Excel version 2000, and used to estimate $s_{0t\,jk}^h$ by (1). The following hypotheses were tested for each of the three sites, by constraining the relationship between parameters in (1): do wild and hybrid plants differ in survival (H_0: $s_{0t\,jk}^w = s_{0t\,jk}^h$)? Do plants from the three maternal populations differ (H_0: $s_{0t\,j1}^i = s_{0t\,j2}^i = s_{0t\,j3}^i$)? Do autumn and spring-sown plants differ ($s_{0t\,1k}^i = s_{0t\,2k}^i$)? Are all F_1 hybrids in the mixed rows dead and only wild plants alive (H_0: $s_{jk}^h = 0$)? Hypotheses were tested by likelihood ratio tests of $D = -2(\ln L_{\text{current}} - \ln L_{\text{saturated}})$, where $L_{\text{saturated}}$ is the maximum likelihood of an unconstrained model and L_{current}

the likelihood of the constrained model; D is approximately chi-square distributed with degrees of freedom equal to the difference in number of variables between $L_{current}$ and $L_{saturated}$ (Hosmer and Lemeshow, 1989). All hypotheses were tested against the full model (including all effects); if a hypothesis was not rejected, the likelihood of its constrained model was used as unconstrained model for subsequent tests.

To estimate and test the probability of flowering, a similar likelihood model was based on the number of flowering individuals, the number of seeds sown, and the unknown parameter $fl^w_{0t\ jk}, fl^m_{0t\ jk}$ and $fl^h_{0t\ jk}$. The same tests as above were made.

To estimate the number and size of F_1 umbels in the mixtures at Amager, we used an approach analogous to that for survival and flowering frequencies. The frequencies of F_1 plants in the mixed rows at time $t = 4$ were estimated from the maximum likelihood survival estimates of wild and F_1 plants. The F_1 frequencies were then combined with the average number and size of umbels in neighboring wild and mixed rows, using equation (1), to estimate the umbel number and size for hybrids. These estimates depend strongly on the correct estimation of frequencies of hybrids in the mixed rows by the likelihood model, however. To test differences in umbel number and size between wild and hybrid plants, we therefore only compared the wild and mixed rows, to be conservative. An ANOVA model, including effects of maternal population, whether or not plants were hybrids, and their interactions were used for these tests. All tests, except for the likelihood model, were made using S-PLUS for Windows (2001).

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