

Population fluctuations, losses to grazing, and reproductive success of *Dactylorhiza sambucina* on Bornholm, Denmark

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Populations of the orchid *Dactylorhiza sambucina* are declining in number and size on the island of Bornholm, Denmark. To study the reasons for this trend, we compiled previously unpublished estimates of population sizes from 1987–2010 and found complicated fluctuations, as population sizes generally increased in the early 1990s but declined after that. We could find no effect of vegetation management, neither were the fluctuations related to suggested climatic factors. High proportions of plants were lost to grazing in the study year; same could be deduced for previous years when more than one census was available. Grazing in spring and early summer may therefore contribute to the decline of *D. sambucina*, as suggested by others. Populations seemed not to be affected by lack of pollination or population inbreeding, as fruit set and reproductive size was as high as in large and interconnected *D. sambucina* populations on Öland, Sweden, and as reported in other studies.

Introduction

Worldwide, many populations of wild plants have declined due to human activities. In Denmark, as in many parts of Europe, a considerable proportion of the landscape once consisted of semi-natural, open habitats, such as meadows, grasslands, and heaths, mainly created through 6000 years of traditional agriculture with extensive grazing and hay harvesting. Today, this use of the habitats has largely been replaced by intensive agriculture. Among the remaining meadows, grasslands and heaths in Denmark, approximately 10% are overgrown due to the lack of grazing and cutting (Buttenschøn 2007). Among the species that are strongly affected by this are

many orchids (Orchidaceae) (Kull & Hutchings 2006, Kull *et al.* 2006, Pedersen and Faurholdt 2010). To conserve their remaining populations it is therefore vital to understand the causes for their decline and how to manage their habitats.

To improve the conditions for a grassland orchid in decline, it may seem obvious that vegetation should be kept open and invading woody species removed, as this will reduce competition especially for light (Pierce *et al.* 2006, Buttenschøn 2007, Sletvold *et al.* 2010). However, not all orchid species respond positively to such efforts, and their population sizes remain low (Pierce *et al.* 2006). *Dactylorhiza sambucina* (Orchidaceae) on the island of Bornholm, Denmark, may be one such example.

Dactylorhiza sambucina is a long-lived and light-demanding orchid (Inghe & Tamm 1988), distributed from southern Scandinavia to central Europe, the Mediterranean, Iran, and at the Black Sea (Nordal & Wischmann 1987, Pedersen and Faurholdt 2010). In Denmark, only 10–14 populations of *D. sambucina* are still surviving, of which the majority are situated on the island of Bornholm in the Baltic sea (*see* www.dmu.dk/dyrplanter/planter/orkideoversigt/). There, the species grows on lime-free acidic soils, and thrives with negligible inputs of nitrogen (Mehl 1985). Its habitats are often humid in winter and spring, but dry up during the summer. *Dactylorhiza sambucina* was earlier fairly common on the island (Grøntved 1948), but its habitats were since then reduced, fragmented and eutrophicated to an extent that the species was in 1985 recognized as endangered (Mehl 1985). Since then active conservation, in the form of extensive grazing, has been implemented at most of these sites. However, it is questionable whether *D. sambucina* populations have responded positively to this, as it still seems to be in decline.

Other factors than vegetation management could affect the survival of these populations. They may suffer from insufficient pollination and thereby inadequate seed production, as population numbers and sizes have declined. *Dactylorhiza sambucina* may be especially vulnerable to this as it is nectarless and needs to deceive inexperienced pollinators to deliver pollen (Nilsson 1980). Pollinators are queens of bumble bees (*Bombus*), they however learn rather quickly that they are deceived, and after visiting few *D. sambucina* plants and flowers, groom themselves to get rid of pollinia (Nilsson 1980). Deceptive orchids therefore depend on abundant numbers of inexperienced bumble bee queens (Nilsson 1980). In fact studies show that *D. sambucina* may be pollinator-limited, with a greater fruit production after hand-pollination than under open pollination (Kropf & Renner 2005, Pellegrino *et al.* 2005). Nectarless species of orchids, like *D. sambucina*, may therefore be especially sensitive to insufficient pollination; indeed there is a predominance of nectarless species among rare orchids in Britain (Neiland & Wilcock 1998). The general decline in bumble bee diversity and abundance in Europe may

further contribute to this (Goulson *et al.* 2008). However, another study of 32 orchid species in Flanders and the Netherlands found no relation between nectar reward and decline in distribution area (Jacquemyn *et al.* 2005).

Genetic erosion and population inbreeding could also contribute to the decline of *D. sambucina*. In general, small and isolated plant populations are genetically less diverse and have a lower vigor and fitness than larger populations (Leimu *et al.* 2006) due to genetic drift and fixation of slightly deleterious alleles (Barrett & Kohn 1991, Hauser *et al.* 1994). Jersakova *et al.* (2006) suggested that *D. sambucina* populations have low genetic connectivity, thereby intensifying the risk of genetic drift and population inbreeding depression (Turner 1989, Dunning *et al.* 1992, Taylor *et al.* 1993, Frankham 2005). Studies have shown that *D. sambucina* may suffer from inbreeding depression upon selfing (i.e. “fast” inbreeding), with lower seed development (Nilsson 1980), germination and survival (Jersakova *et al.* 2006, Juillet *et al.* 2007). This indicates that even with a slower progress of inbreeding, as in small isolated populations, the genetic load may cause a decrease in vigor and fitness.

Here, we studied the fluctuations and decline of the remaining *D. sambucina* populations on Bornholm (Denmark) and whether grazing, climate, lack of pollination and population inbreeding contribute to this. We collected information on population sizes from several unpublished sources to clarify how these responded to vegetation management by grazing and other forms of clearing, and to a number of climate variables. We further asked whether insufficient pollination and population inbreeding affects *D. sambucina* on Bornholm, by comparing a number of reproductive and size traits between plants on Bornholm and Öland (Sweden), which harbors large and interconnected populations of *D. sambucina*. From our results, we discuss future management of the *D. sambucina* habitats.

Material and methods

Dactylorhiza sambucina populations

The extant populations of *Dactylorhiza sambu-*

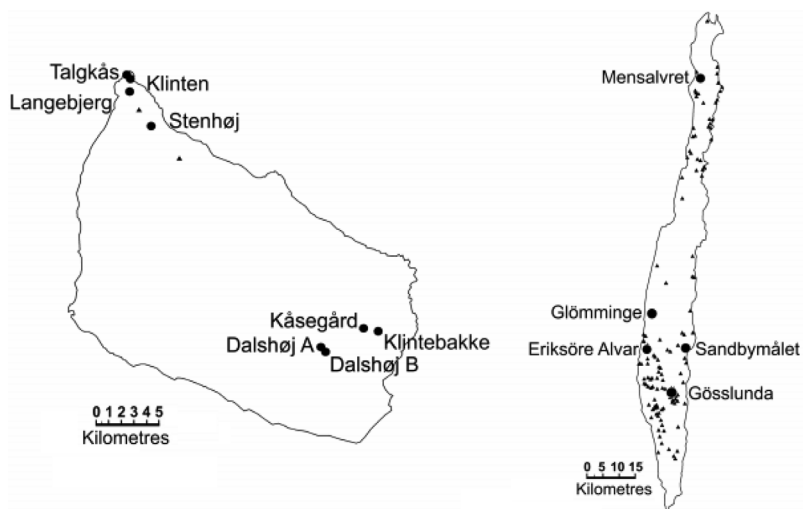


Fig. 1. Known extant *Dactylorhiza sambucina* populations on Bornholm, Denmark, and Öland, Sweden (triangles), and populations included in the present study (dots).

cina on Bornholm are situated in two clusters, approximately 25–30 kilometres apart (Fig. 1). Eight of these were included in our study; one of the remaining populations is expected to be lost (Trönhøj), another (Store Myregård) might still exist as a small population but knowledge about it is scarce. Populations are generally small and isolated; most of them have been grazed or managed in other ways especially in the last 10–15 years (see Fig. 2 for a summary of the management methods). Unlike Bornholm, Öland has more than 100 *D. sambucina* populations containing at least 10 000 individuals, mainly situated in the southern part of the island (Fig. 1; U.-B. Andersson pers. com.). Five of the Öland populations were chosen for our study of pollination and inbreeding effects, based on occurrence, recommendations from J.C. Schou (pers. comm.), nature types, conservation regimes, and an even collection throughout the region. All populations grow on alvar — a limestone habitat with thin soil and sparse vegetation — except in Glömminge (calcareous grassland), and are managed by grazing or hay harvesting.

Population size fluctuations

To improve knowledge on the sizes and fluctuations of the Bornholm populations, we compiled three data sets for the period 1987–2010. The largest data set was available from the National Envi-

ronmental Research Institute (www.dmu.dk/dyrplanter/planter/orkideoversigt/), which was complemented with the data collected by O.H. Pedersen and J. Christensen (pers. comm.). These data were compared with the known vegetation-management data of the same populations (Fig. 2), compiled from information provided by O.H. Pedersen, T.H. Bjerregaard and L.L. Bojesen, and from Bojesen *et al.* (2005), to analyze whether management had a positive effect on sizes. In addition, we analyzed if population fluctuations correlated with monthly and annual mean, maximum and minimum temperatures; mean daily maximum and minimum temperature; precipitation and hours of sunshine in Denmark (Cappelen & Jørgensen 2010). Time series of weather data were inspected (all months together, and subsets for winter, spring and summer months), and correlated with the population trends.

Plant size and reproductive traits

To test whether the populations were affected by pollination deficit and population inbreeding, all plants in the eight Bornholm populations were counted in late June 2010, and plant and inflorescence height, number of flowers and developed fruits, and fruit diameter were measured (sample sizes in Fig. 3). At that time, flowering had finished and the fruits were ripening; developing and non-developing fruits could easily be differ-

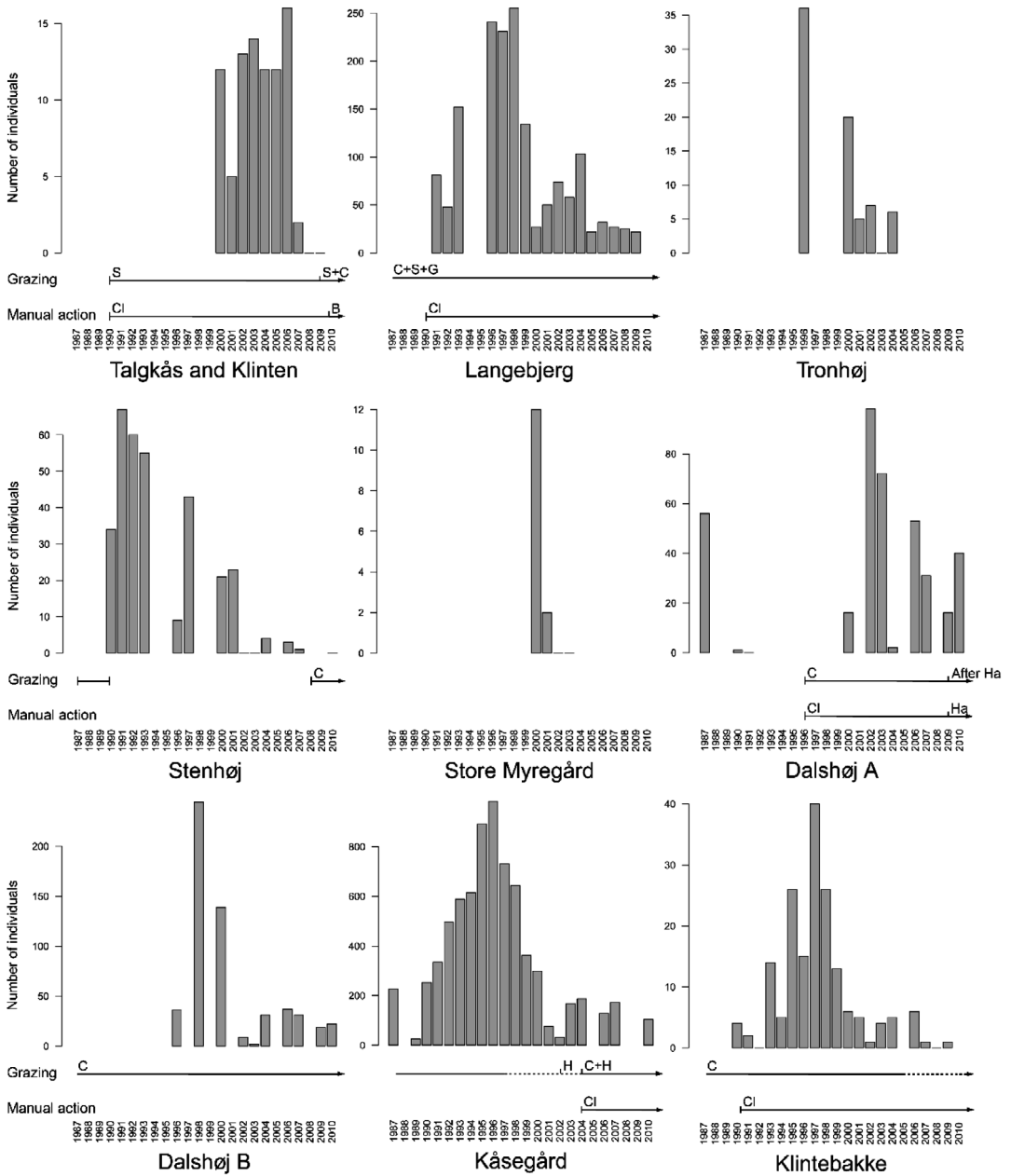


Fig. 2. Population sizes (average if more than one count was available) and management of nine *Dactylorhiza sambucina* populations on Bornholm, compiled from different sources (see text). Vegetation management is indicated as follows: grazing by cattle (C), sheep (S), goats (G), or horses (H); burning (B), clearing of brush (Cl), or hay harvesting (Ha). Dotted lines indicate low intensity. Note different scales of the y-axes. Populations Talgkås and Klinten were treated as one in the historical censuses, Dalshøj is treated as two populations/patches, Tronhøj is probably extinct, and there is no knowledge on the status of Store Myregård from recent years.

entiated. Populations on Öland were visited in early July 2010; the numbers of flowering individuals were estimated visually, and 30–60 ran-

domly chosen flowering individuals measured as above. In mid-July, Langebjerg, Dalshøj B and Kåsegård were revisited to see if additional

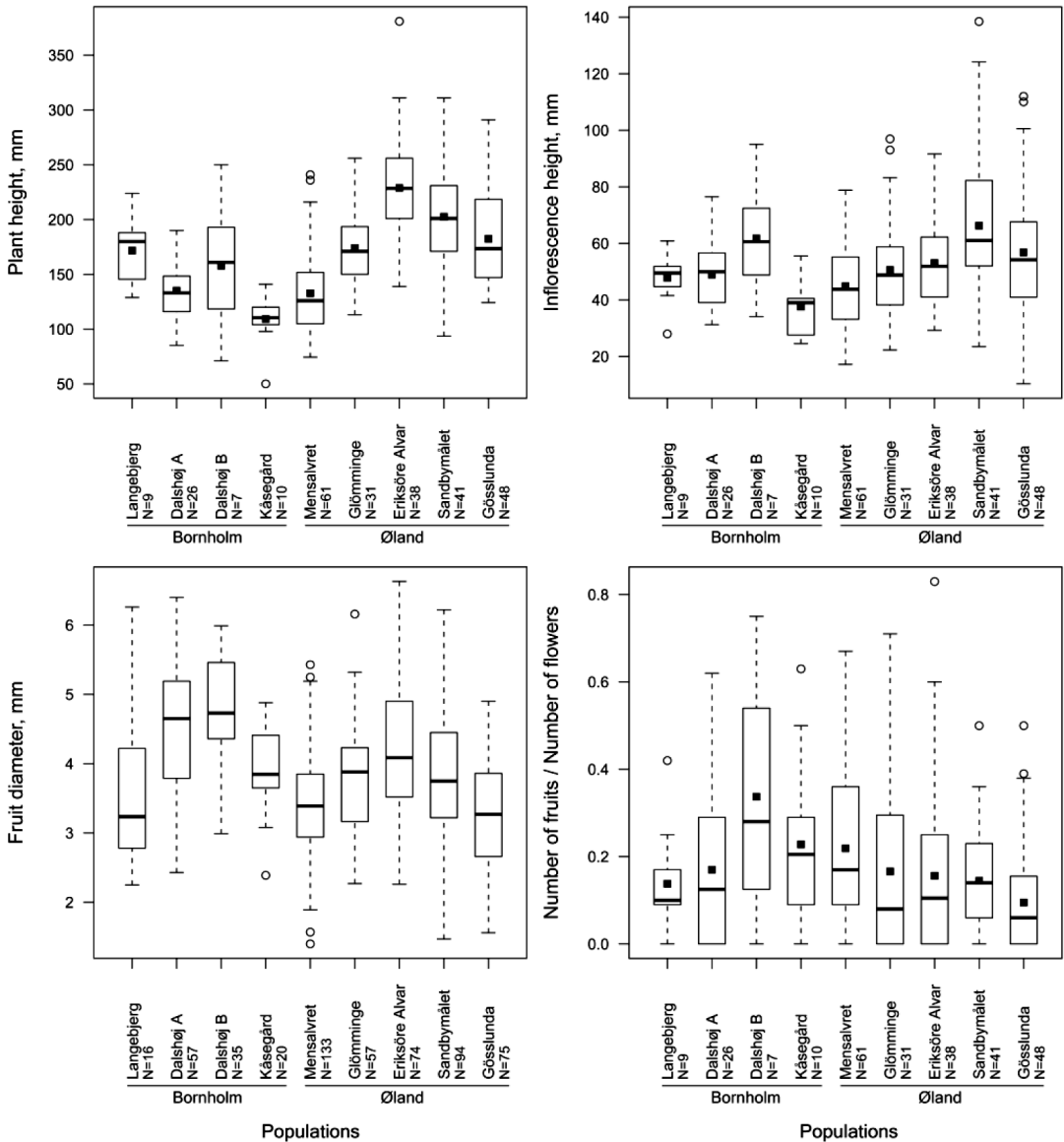


Fig. 3. Plant and inflorescence height, fruit diameter and fruit: flower ratio in *Dactylorhiza sambucina* populations on Bornholm and Öland. The boxplots show the 10, 25, 50, 75, and 90 percentiles, in addition to outliers (circles) and means (black squares).

plants could be discovered, and to test if fruit diameter had changed since the first visit.

At this time, we observed that a substantial number of plants had been lost since the first census in June, due to animal grazing; we therefore recounted the number of plants. We could further quantify losses to grazing, as we got access to counts of flowering individuals from May of the same year from O.H. Pedersen (pers. comm.).

Differences in plant height and fruit diameter between populations on Bornholm and Öland were tested with nested ANOVA, using 'location' and 'population' as grouping variables, in addition to 'plant' for the fruit data. Differences in inflorescence height were analyzed using a Kruskal-Wallis rank-sum test, as it did not fulfill assumptions of ANOVA, using the same nesting levels as for plant height. From the counts of flowers and fruits per plant we estimated the pro-

portions of flowering plants that set fruits, and the proportions of flowers that developed into fruits (on individuals with fruits). Differences in these estimates between Bornholm and Öland were tested by one-way ANOVA of log-transformed proportions, followed by a pairwise *t*-test with Holm's correction for multiple testings. To further test if population sizes had an effect on plant height, inflorescence height, fruit diameter, and fruit: flower proportion, correlations between the mean for each of these traits and population sizes were examined, using Pearson's (plant height and fruit diameter) and Spearman's correlations (inflorescence height and fruiting proportion). For Bornholm, population sizes for correlations were estimated based on the data on number of flowering plants in May 2009 and 2010 from O.H. Pedersen (pers. comm.). On Öland, population sizes were estimated based on the number of flowering individuals in July 2010.

We could additionally compare if plant height, inflorescence height, and number of flowers had changed over time in Langebjerg and Kåsegård, by combining our own data with data from 1999 kindly provided by H.Æ. Pedersen (from Pedersen 2006). ANOVA, a Wilcoxon rank-sum test, and a Generalized Linear Model with quasi-Poisson distribution were used for these analyses, respectively. All statistical analyses were carried out in R (2010).

Results

Population fluctuations, management and climate

Our compiled data on population sizes on Bornholm from 1987 to 2010 show that the *D. sambucina* populations declined in recent years (Fig. 2). However, the population sizes fluctuated strongly during this period, and especially pronounced was a local optimum in 1996–1998 in several of the populations, before which they were smaller, some of them even as small as they are today.

There was no clear relation between population fluctuations and methods of vegetation management at the sites. In several of the populations

(Langebjerg, Dalshøj B, Kåsegård and Klintebakke), size increased before and decreased after 1996–1998, even though the management methods remained the same (Fig. 2). Thus, their decline started even under grazing and brush clearing. In Dalshøj A and Kåsegård, however, there is an indication of a slight improvement during the latter years, but this is not entirely clear yet. We did not find any relation between population size fluctuations and climatic conditions.

Losses to grazing

Many flowering individuals on Bornholm were found to be lost to grazing when revisiting the populations at Langebjerg, Dalshøj B and Kåsegård a second time in July. Some plants had disappeared completely; others still had remains of the flowering stem. Using the additional data of O.H. Pedersen (pers. comm.) from May, it was evident that substantial proportions of plants had disappeared from the four sites between May and July (Fig. 4); as an extreme, more than 90% of the flowering plants had been lost at Kåsegård; Langebjerg and Dalshøj had lost more than 50%. Dalshøj A was mowed before the late visit; still it lost about a third of its plants between May and our first count.

Plant losses were also suggested from the data on population sizes compiled over time: counts were available from more than one source for some years, and the late counts could indicate a loss of almost 20 individuals. As *D. sambucina* is very conspicuous when flowering, these discrepancies are likely caused by loss of individuals to grazing in between counts.

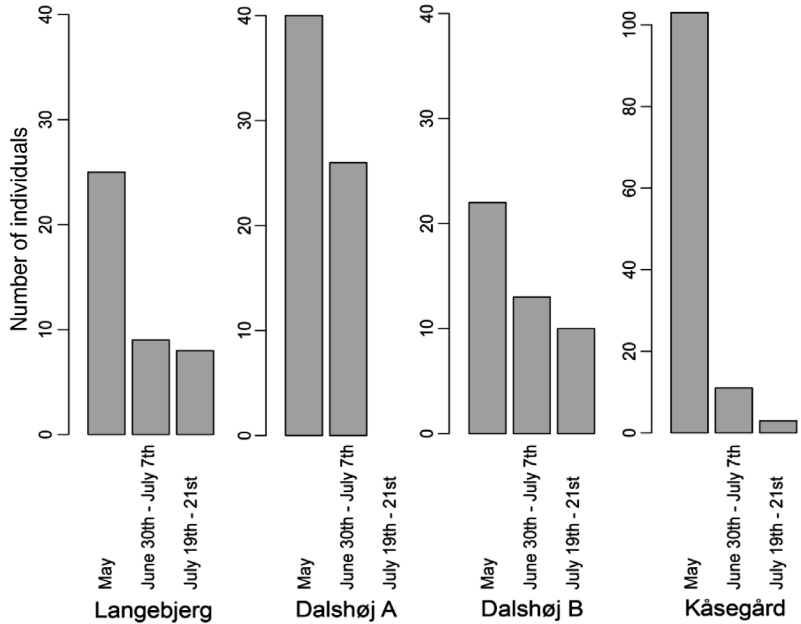
Losses of plants on Öland could also be considerable, as several damaged individuals were observed during our visit.

Plant vigour and reproductive success

No flowering individuals were found in the populations Talgkås, Klinten, Stenhøj and Klintebakke on Bornholm in June 2010, hence the following applies only to the other populations.

The plants on Öland were taller than those

Fig. 4. Losses of plants during the 2010 flowering season, as indicated by counts of flowering individuals in four Bornholm populations at three different times. Data from May were provided by O.H. Pedersen (pers. comm.), those from June and July were counted as part of this study. Dalshøj A was harvested for hay in the middle of June, and therefore not revisited. Note different scales of the y-axes.



on Bornholm (Fig. 3; nested ANOVA: $F_{1,261} = 479, p < 0.001$), but apart from this there were no systematic differences between the plants from the two locations in inflorescence height (Fig. 3), number of flowers and fruits per plant (Table 1), proportion of plants that set fruits, proportion of flowers that developed into fruits (on individuals with fruits), or diameter of fruits (Fig. 3; non-significant effects of locality in nested or one-way ANOVA). Approximately 70% of the measured individuals developed fruits, and approximately 10%–20% of the flowers developed into fruits.

Neither plant or inflorescence height, nor fruit-to-flower ratio correlated with population sizes (Pearson or Spearman correlations, $p > 0.05$), while fruit diameter was negatively correlated (Pearson's product-moment correlation: $r = -0.62, p < 0.05$).

When comparing our data from 2010 with those from 1999 (Pedersen 2006), neither plant height, inflorescence height, nor the number of flowers changed over time at Langebjerger (non-significant effects of year in one-way ANOVA). At Kåsegård, however, the plants were 35.6% lower in 2010 than in 1999 ($F_{1,36} = 32, p < 0.001$), the inflorescences shorter ($W_{1,36} = 221, p = 0.007$) and contained 31% fewer flowers ($t_{1,36} = -2.8, p = 0.008$).

Discussion

Population fluctuations and vegetation management

Populations of *Dactylorhiza sambucina* have clearly declined in number and size on the island of Bornholm, as well as in the rest of Denmark (Grøntved 1948, Mehl 1985). However, the data we compiled show that they did not decline

Table 1. Mean numbers (n , SE) of flowers and fruits per plant in *Dactylorhiza sambucina* populations from Bornholm (Denmark) and Öland (Sweden).

	Number of flowers per plant	Number of fruits per plant
Bornholm		
Langebjerger	11.0 (9, 2.1)	1.56 (9, 1.59)
Dalshøj A	10.6 (26, 3.2)	1.88 (26, 1.99)
Dalshøj B	12.3 (7, 3.9)	3.86 (7, 2.79)
Kåsegård	8.3 (10, 2.9)	1.80 (10, 1.55)
Öland		
Mensalvret	9.7 (61, 2.9)	1.80 (61, 1.55)
Glömminge	8.5 (31, 3.3)	1.32 (31, 1.51)
Eriksöre Alvar	9.2 (38, 2.3)	1.50 (38, 1.97)
Sandbymålet	15.6 (41, 6.2)	2.10 (41, 1.76)
Gösslunda	12.2 (48, 4.7)	1.10 (48, 1.45)

gradually during the last 25 years. Four out of the nine populations, for which we could obtain data, had increased to a temporary optimum in 1996–1998 and declined thereafter. Other populations had temporary maxima earlier or later in the period. These fluctuations are not related to changes in vegetation management. The populations at Langebjerg, Dalshøj B and Klintebakke increased before 1996–1998, but decreased after that, even though vegetation management did not change during the whole period. The same holds for Dalshøj A, which had a maximum in 2002–2003. Kåsegård decreased from 1997 to 2004 coinciding with a low intensity of grazing; however, it has responded only little to reestablishment of grazing and clearing in 2004. Similarly, Stenhøj declined after grazing stopped, but has not increased after grazing was reintroduced in 2008–2010. This lack of response to improved management may be due to slow seed germination of orchids (Rasmussen & Whigham 1993, Arditti & Ghani 2000) and long time of development to mature plants (Kery & Gregg 2004, Sletvold *et al.* 2010). For example *D. incarnata* only responded to resumed management within five years (Reinecke 1988).

It is indisputable that *D. sambucina* requires low and open surrounding vegetation, and that the vegetation therefore needs to be managed. Our results, however, indicate that the management of the *D. sambucina* sites at Bornholm may not have been optimal, or that other factors have influenced the dynamics and recent decline of the populations.

Losses to grazing

A surprisingly high proportion of *D. sambucina* plants disappeared between early spring and the middle of the summer, most likely because of grazing. More than 90% of the flowering individuals disappeared from one of the populations on Bornholm, and more than half from two others of the four populations we could inspect. Damaged individuals were observed on both Bornholm and Öland, and losses to grazing were further suggested by historical data, when counts were available from more than one source. Given the small population sizes and conspicuous inflo-

rescences, it is unlikely that such differences are due to counting errors. Negative effects of grazing on *D. sambucina*, especially by sheep and goats, have also been suggested in previous publications (Kjøller 1982, Pettersson & Nilsson 1983, Norderhaug *et al.* 1997), and also in publications on other orchid species (Tamis *et al.* 2009, Alexander *et al.* 2010). On Öland, reintroduction of grazing in sites that had been unmanaged for some years improved the frequency of *D. sambucina* (Rosen & Bakker 2005); however, sheep and cattle were found to selectively graze on *D. sambucina*, which may cause problems for its persistence (Rosen & Bakker 2005). Horses have been suggested as “better” grazers of orchid habitats, as they avoid e.g. *Dactylorhiza* plants; however, this is not always the case (*see* Darinot & Morand 2001).

Losses of individuals and their fruits and seeds may potentially affect especially small and isolated *D. sambucina* populations severely. A study of defoliation of *D. sambucina* showed that a complete loss of leaves caused an elevated proportion of tubers that remained dormant during the following season (Pellegrino & Musacchio 2006). Among the individuals that did produce shoots, higher proportions than average had a reduced leaf area, grew vegetatively, and produced fewer fruits. Grazing of *D. sambucina* may thus decrease seed production and ultimately regeneration of the population. This may have an especially severe impact for a species like *D. sambucina*, where regeneration is slow and death rate of seedlings high. Surviving seedlings spend up to 12 years from their first appearance until first flowering (Tamm 1948, 1972, Inghe & Tamm 1988), and individuals can remain vegetative for more than 10 years between flowering (Tamm 1948, 1972, Inghe & Tamm 1988). A lower number of flowering individuals may lead to decreased attraction to pollinators and increased loss of genetic variation.

Insufficient pollination or population inbreeding depression?

If the small and isolated *D. sambucina* populations on Bornholm were not visited by sufficient pollinators, we would expect a lower propor-

tion of individuals bearing fruits, and a lower proportion of flowers that developed into fruits than in the large and open populations on Öland. However, the proportion of individuals developing capsules was not different between the two regions. About 70% of the flowering individuals on Bornholm bore fruit, which is at the high end of what was reported by Kropf and Renner (2005; 9.54% to 78.05%). Likewise, the fruit-to-flower ratio did not differ between Bornholm and Öland, either; actually Dalshøj B on Bornholm had a higher fruit-to-flower ratio than Gös slunda and Sandbymålet on Öland. The overall fruit-to-flower ratio was about 17% for the populations, which is within the range reported in other studies (2.0% to 56.1%; Nilsson 1980, Pettersson & Nilsson 1983, Neiland & Wilcock 1998, Kropf & Renner 2005). Thus, it seems that *D. sambucina* is able to attract sufficient pollinators.

If the populations on Bornholm were negatively affected by population inbreeding, due to increased homozygosity of slightly deleterious alleles from genetic drift, we could expect the plants to be smaller and/or reproductively less successful than in the large, presumably non-inbred populations on Öland (Hauser *et al.* 1994, Leimu *et al.* 2006). However, the plants on Bornholm did not differ from those on Öland in the proportions of plants and flowers that developed fruits (*see above*) or in fruit diameter, measured as a proxy for the number of seeds. In some Öland populations, plants were higher than on Bornholm; however, many of those grew underneath *Prunus spinosa* where they were sheltered from grazers and probably had “stretched” for light. The plant height on Bornholm was close to what was reported by Kropf and Renner (2005; 16 cm). Neither did we find a negative correlation between population size and the measured traits, nor a negative change from 1999 to 2010 at Langebjerg, despite a drop in population size from 134 to only 9–24 flowering individuals in that period. An exception may be the population at Kåsegård, where the plants were smaller and produced fewer flowers in 2010 than in 1999; this population decreased from 362 to 103 individuals. However, in 1999 the plants were measured in May (Pedersen 2006) whereas in 2010 they were measured in July on plants that had survived grazing by cattle

and horses. They may have survived preferentially due to their small size, as found for other orchids (Alexander *et al.* 2010).

Hence, our results suggest that the populations on Bornholm are not negatively affected by population inbreeding, despite their present small population size and isolation. Likely, too few generations have passed since the populations were larger and more wide-spread, too few for genetic drift to decrease the genetic variation and build up inbreeding. Gene flow with neighboring populations may also be maintained to some extent, as the dust seeds of orchids can disperse over long distances (Arditti & Ghani 2000). Alternatively, the plants may be affected by inbreeding in other traits than measured here. Fast inbreeding by selfing in *D. sambucina* results in seeds without embryos (Nilsson 1980), seeds that do not germinate (Jersakova *et al.* 2006), or seeds and seedlings that do not survive (Juillet *et al.* 2007); this may suggest that slow population inbreeding, as expected for genetic drift in small populations, may also affect these traits, which we did not measure. Likewise, inbreeding depression has been found in several other nectarless orchid species for seed and later life stages (Lammi *et al.* 2003, Smithson *et al.* 2007).

Other causes of population fluctuations

Since the population fluctuations of *D. sambucina* on Bornholm did not seem to be related to changed vegetation management, lack of pollination or population inbreeding, weather conditions could be an explanation. *Dactylorhiza sambucina* benefits from humid springs and dry summers, but is negatively affected by early-summer drought (Nordal & Wischmann 1987, Inghe & Tamm 1988). Additionally, studies of other orchid species have indicated that especially harsh winters affect plant growth and fruit production of the following seasons (Pfeifer *et al.* 2006, Hutchings 2010). However, we did not find any correlation between population fluctuations and winter weather or spring and summer precipitation. The winter was harsh in 1996 but mild in 1997 (Cappelen & Jørgensen 2010) when the *D. sambucina* populations peaked; spring and summer in 1996 were quite dry whereas

the summer of 1998 had a lot of precipitation. In contrast, the winters in 1990 and 1991 were mild, but the numbers of flowering plants were low.

Fluctuations could also result from natural variation in emergence and flowering. Several studies on *D. sambucina* show great inter-annual and inter-population variation in the proportions of individuals that emerge, and in the proportions of vegetative and flowering plants (Nilsson 1980, Tamm 1991, Kropf & Renner 2005). Whereas dormancy in tubers only seems to last for one year at a time, *D. sambucina* individuals may remain vegetative for more than 10 years between flowering (Inghe & Tamm 1988). Since the data for population sizes on Bornholm nearly exclusively include numbers of flowering individuals, the exact population sizes are unknown. Thus, the decline over the last 10–15 years might to some extent be an expression of variation in the proportion of vegetative and flowering shoots, rather than a true population decline. Similarly, a study of population size variations in *Orchis morio* found that only demographic studies lasting several decades could determine that a population has declined (Gillman & Dodd 1998).

Other biotic and abiotic changes may have affected the populations of *D. sambucina* on Bornholm negatively. Among them, eutrophication and the resulting changes in vegetation composition and competitive conditions is important, as it is for a number of other plant species in open low-nutrient habitats.

Implications for conservation of *Dactylorhiza sambucina*

Our results on losses of *D. sambucina* to grazing animals suggest that management of the populations could be improved, either by reducing, shifting, or abandoning grazing in May and July. By mid-July, when the fruits are ripe, grazing can be increased again. This would mimic the historical uses of *D. sambucina* meadows on Bornholm (Mehl 1985), where hay was harvested in mid-summer, after which the meadows were grazed. Several other studies support that late hay harvesting and grazing may be the optimal management method for these orchids

(Inghe & Tamm 1988, Tamm 1991, Norderhaug *et al.* 1997, Kropf 2008); similar recommendations have been made for other grassland orchids (Janeckova *et al.* 2006, Sletvold *et al.* 2010). Later in the summer, even partial abandonment of grazing is harmful for *D. sambucina* (Inghe & Tamm 1988, Tamm 1991). Other studies on orchids have shown that the plants may be strongly affected by different timing (Janeckova *et al.* 2006, Sletvold *et al.* 2010) and frequency of management (Janeckova *et al.* 2006, Schleuning & Matthies 2009, Sletvold *et al.* 2010, Schrautzer *et al.* 2011). The timing of mowing and grazing may therefore be almost as important for *D. sambucina* as mowing and grazing *per se*.

Another implication of our results on grazing is that population censuses may be highly affected by their timing. Very different population sizes would have been obtained at different times in the spring of our study, with much smaller numbers of countable individuals later in the season (*see* Fig. 3).

Even though we did not find indications of a pollination deficit or population inbreeding effects, most of the populations are of a size where these effects may affect their vigor in the future. We can therefore hope that the future management efforts will increase the sizes and connectivity of the *D. sambucina* populations on Bornholm.

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