Acta Bot. Neerl. 42(1), March 1993, p. 81–91

Geographical variation in threshold size for flowering in *Cynoglossum officinale*

R. A. WESSELINGH, T. J. DE JONG, P. G. L. KLINKHAMER, M. J. VAN DIJK and E. G. M. SCHLATMANN

Department of Evolutionary and Ecological Sciences, University of Leiden, P.O. Box 9516, 2300 RA Leiden, The Netherlands

SUMMARY

We investigated variation in two traits that determine generation time, cold- and size-requirement for flowering, within and among European populations for the monocarpic perennial *Cynoglossum officinale*. When grown in an experimental garden in Leiden, no annual individuals were found among plants originating from 22 locations; all plants were biennial under nutrient-rich growing conditions. In a controlled-environment experiment, in which plants received an artificial cold treatment, flowering probability increased gradually with plant size for plants from two natural populations, signifying a large within-population variation in threshold size for flowering. The relationships between plant size and flowering of these two groups were significantly different: plants from Holkham (England) had much higher threshold sizes than plants from Meijendel (The Netherlands). Three plant groups originating from botanic gardens showed a sharp increase in flowering probability with size, indicating less variation in threshold size. Significant differences existed among all five groups. Results indicate the possibility that natural selection acts upon threshold size for flowering in *Cynoglossum officinale*.

Key-words: Cynoglossum officinale, geographical variation, life-history variation, monocarpy, threshold size for flowering, vernalization.

INTRODUCTION

The life-history strategy of an organism is an important factor in determining its fitness (Stearns 1976). In the past decades attempts have been made to show natural selection acting on life-history traits. Many of these have been carried out on animals, for instance the experimentally induced life-history variation in guppies (Reznick *et al.* 1990). Measuring lifetime fitness in plants is a major practical problem (Primack & Kang 1989), which makes it difficult to study plant life histories.

Monocarpic plants have only one reproductive bout, and are therefore suitable objects for studies on life-history tactics and fitness. For these plants the concept of size at first reproduction is particularly important, because the timing of a single act of reproduction is crucial in determining reproductive success. For annual plants, the relevant strategy concerns the timing of flowering within a season.

In monocarpic perennials ('biennials') lifetime is very variable. The relevant question here is in which year or at what size an individual should reproduce, thereby determining both seed production and generation time. Many studies have shown that this 'decision' is based on size rather than age. Flowering at a small size implies a short generation time, but also a relatively small seed production, due to the close relationship between plant size and seed production found in many monocarpic perennial species (e.g. *Cynoglossum officinale* L.: Klinkhamer & De Jong 1987). Flowering at a larger size implies a longer generation time and an increased probability of dying before the flowering stage is reached, but the advantage of a higher seed production. Furthermore, spreading the reproduction of the progeny over different years may be profitable in strongly variable environments with a low juvenile survival (Klinkhamer & De Jong 1983; Van der Meijden *et al.* 1992).

Most monocarpic perennial plant species need an external trigger for flower induction, usually winter cold and/or long-day conditions. These requirements are not always absolute: in a number of species individuals have been found that flowered without cold. Plant size determines whether a plant will react to the flowering stimuli or not. In many species a minimum size—also called critical size—for flowering can be found (Werner 1975; Baskin & Baskin 1979a,b; Van der Meijden & Van der Waals-Kooi 1979; Gross 1981; Hirose & Kachi 1982; Gross & Werner 1983; Lee & Hamrick 1983; Augspurger 1985; De Jong *et al.* 1986; Klinkhamer *et al.* 1987a,b; Prins *et al.* 1990; Klinkhamer *et al.* 1991). In a number of these papers authors write about 'the' minimum size, suggesting that this size is the same for all plants in a population. However, in almost all of these studies it is not possible to pinpoint one single size that divides the population into flowering and non-flowering plants. Most data sets even show a large overlap in plant size of flowering and non-flowering individuals. This is not only the case when authors measured plants during the flowering season, when size differences can be the result of different growth rates in spring (Baskin & Baskin 1979b; Lacey 1986), but also when plant sizes just before winter are compared (De Jong *et al.* 1986). Apparently, individual plants within populations have different minimum sizes. To avoid semantic confusion we will use 'threshold size' for the size an individual plant has to reach to be able to flower. We will use 'minimum size' for the smallest threshold size found in a population. Plants smaller than their individual threshold size do not react to flowering stimuli, even if this size is well beyond the minimum size in the population.

So within-population variation—genetic or phenotypic—in threshold size for flowering seems to be quite large. De Jong *et al.* (1989) developed an optimization model based on field data for *Cynoglossum officinale* and *Cirsium vulgare* (Savi) Ten., in which they demonstrated that the population rate of increase λ is fairly equal for lineages with threshold sizes larger than the optimal threshold size (*c.* 2 g). Below this size λ drops very rapidly. So selection will be strong only on threshold sizes smaller than optimal, and a large variation in threshold sizes can be maintained in a population. Indications for a genetic basis for threshold size are given by a study on *Daucus carota* L. (Lacey 1986). Annual mothers produced the greatest number of annual offspring, and offspring of triennial plants showed the strongest tendency to delay flowering beyond the second year.

Our knowledge of the variation among populations is much more restricted. In a common-garden experiment with *Verbascum thapsus* L. in North America (Reinartz 1984), delay of flowering until the third year was most common among northern genotypes. Annual plants were found among southern genotypes only. Lacey (1988) found the same pattern in delay of flowering for *Daucus carota* in eastern North America. For

Cirsium vulgare, annual genotypes were found in populations from southern Europe (Klinkhamer & De Jong 1993).

No attempt has yet been made to relate these differences in generation time to actual threshold sizes. Insight into the range of threshold sizes found in different populations is needed in order to elucidate the function of threshold size. A prerequisite for selection on size at first reproduction is the presence of genetic variation for threshold size within a species (Endler 1986; Kachi 1990). The same can be said about variation in cold requirement: only the presence of genotypes lacking the cold requirement makes annual life-histories possible. The genetic background of threshold size for two perennial monocarps is currently being investigated at our laboratory. The purpose of this paper is to show the presence of variation, both within and among populations, in cold requirement and threshold size for flowering in *Cynoglossum officinale* L.

MATERIALS AND METHODS

Cynoglossum officinale (Boraginaceae) is an insect-pollinated, monocarpic perennial that is widely distributed throughout Europe, from 68°N in Sweden down to the Mediterranean region (De Jong *et al.* 1990). It is a species of open vegetations on calcareous soils. We have studied this species for 10 years in Meijendel, a sand-dune area near The Hague, The Netherlands, where it shows a monocarpic perennial life-history. Plants from Meijendel show a straightforward reaction to flowering stimuli: if the threshold size has been exceeded at the onset of winter, plants form flower primordia during the winter period. Plant age, plant nitrogen concentration, and light intensity before the cold period have no effect on flowering probability when corrected for size differences. No effects of conditions after winter on flowering probability have been detected (De Jong *et al.* 1986). This excludes the possibility of variation in generation time in common-garden experiments as a result of different light, temperature, or nutrient requirements after winter.

Experiment 1: variation in cold requirement

Seeds from 22 sites were either collected in the field or obtained from botanic gardens from different regions in Europe (Table 1). Seedlings were planted in an experimental garden in Leiden on 14 April 1986, after stratification (dark, 5°C) and germination (15°C/5°C, daylength 12 h) of the seeds in the laboratory. Because of the nutrient-rich soil, plants could grow quite large in their first growing season, so expected threshold sizes for flowering could easily be exceeded before winter. Estimated root dry weights at the end of May were > 10 g. Date of bolting and date of opening of the first flower were recorded.

Experiment 2: variation in threshold size

Two natural populations were chosen to study the relationship between plant size and flowering probability: Meijendel (The Netherlands) and Holkham (England). Both sand-dune areas are similar in soil type, vegetation, and climate. The *Cynoglossum* plants grow most often in habitats with a low vegetation cover. The most notable difference between the plants in Meijendel and Holkham was the occurrence of repeated flowering in Holkham. In an experimental garden 16% of the plants behaved as short-lived perennials: they flowered twice, in two consecutive seasons (Boorman & Fuller 1984). In Meijendel repeated flowering occurs only very rarely, and presumably only after damage to the root crown (De Jong *et al.* 1990).

Table 1. Locations of the *Cynoglossum officinale* sites of seed collection. Indicated are the (approximate) coordinates of the site, if the origin of the seeds was wild (w) or in a botanic garden (b), the number of plants grown, and the average date of flowering in the second year. No plants flowered in their first year

Site	Coordinates		Seed origin	*n*	Date of flowering ±SD (days)
Oulu, Finland	65°00′N	25°26′E	b	6	20 May±3
Turku, Finland	60°27′N	22°15′E	b	6	25 May±3
Riga, Latvia	56°53′N	24°08′E	b	3	15 May±4
Hälsingborg, Sweden	56°05′N	12°45′E	b	5	2 June±2
Holkham, England	52°58′N	00°48′E	w	6	27 May±3
Warszawa, Poland	52°15′N	21°00′E	b	5	24 May±3
Stroe, Netherlands	52°11′N	05°41′E	w	2	12 May±0
Meijendel, Netherlands	52°05′N	04°16′E	w	5	12 May±5
Oostvoorne, Netherlands	51°55′N	04°06′E	w	6	17 May±4
Lódź, Poland	51°49′N	19°28′E	b	7	16 May±4
Cardiff, Wales	51°30′N	03°13′W	b	5	21 May±3
Tegelen, Netherlands	51°21′N	06°08′E	b	5	17 May±6
Düsseldorf, Germany	51°13′N	06°47′E	w	6	27 May±1
Bemelen, Netherlands	50°50′N	05°45′E	w	5	23 May±2
Gembloux, Belgium	50°34′N	04°42′E	b	6	15 May±2
Kiev, Ukraine	50°25′N	30°30′E	b	1	22 May
Kosice, Czechoslovakia	48°44′N	21°15′E	b	5	12 May±0
Budapest, Hungary	47°30′N	19°03′E	w	7	7 May±4
Bern, Switzerland	46°57′N	07°26′E	b	6	20 May±8
Champex, Switzerland	46°02′N	07°08′E	w	5	18 May±3
Ljubljana, Slovenija	46°04′N	14°30′E	b	3	18 May±6
Bordeaux, France	44°50′N	00°34′W	b	6	16 May±3

Three additional seed sources, all from botanic gardens, were used to examine differences between natural and cultivated populations. In Oulu and Turku (Finland) the populations consisted of a small number of plants (5–10) in the local botanic gardens. These small populations had been in cultivation for at least 10 generations. The seeds from Bordeaux (France) also originated from a botanic garden, but this population was larger, about 50 plants. The duration of cultivation was unknown.

Starting in December 1986, seeds were kept for 58 days at 5°C in the dark in Petri dishes on moist filter paper for stratification. In February 1987 the seeds were transferred to warmer conditions (12 h light at 15°C, 12 h dark at 5°C) for germination. Sixty-three seedlings per population were planted in containers (height 18 cm, diameter 12 cm, capacity 1·5 litre) filled with a 1:1 mixture of dune sand and compost. The plants were placed in a growth room in a randomized design, with 16 h light (35 W m^{-2}, provided by Philips lamps type SON-T 400 W) at 25°C and a night temperature of 15°C. Standard Hoagland nutrient solution was supplied, starting with 75 ml in week 2 after planting and gradually increasing to 250 ml in week 8. To obtain plants of different sizes during the cold period 10 plants per population were transferred to a cold room each week, starting in week 4. Just before the transfer, number of leaves (living and dead) and the length of the two longest leaves were corded as a non-destructive measure of plant size. All 250 plants stayed in the cold room (8 h light, 7 W m^{-2}, 4°C) for 8 weeks. Previous experiments had shown that 6 weeks at 4°C was sufficient for flower induction (De Jong *et al.* 1990). Two

plants, both from Holkham, died during this period. After the cold treatment, plants were transferred to a growth room (16 h light, 15°C, 35 W m^{-2}; 8 h dark, 10°C) and kept there for 6 months after the cold treatment. Plants that did not flower within this period were considered to be non-flowering.

The remaining 13 plants per population were measured and harvested at three different moments during the experiment to obtain a relationship between the non-destructive size measures and the actual dry weight. Total number of leaves (*TNL*), alive and dead, was recorded, and the length of the two longest leaves (*LLL*) measured to the nearest millimetre. Plants were divided into shoots and roots. Only the tap roots—the bulk (>90%) of the root biomass—could be harvested: the smallest roots were too much entangled in the compost to be separated. All parts were dried at 70°C and weighed to the nearest 0·01 g. The roots comprised 34% of the total dry weight (shoot–root ratio 1·9), which lies in the normal range for this species when grown under nutrient-rich conditions (De Jong *et al.* 1990). From these data we derived a relationship to estimate plant dry weight (*DW*, g) for the experimental plants:

$$DW = 0{\cdot}93 + 6{\cdot}27 \times 10^{-4} \times TNL \times LLL \ (\mathrm{df} = 64, r^2 = 0{\cdot}834).$$

Statistical analysis. To quantify the relationship between plant weight and the probability of flowering we performed a maximum likelihood analysis on the data, as described in more detail by Klinkhamer *et al.* (1987a, 1991). It is assumed that the probability of flowering for the *i*th plant (p_i) is described by:

$$p_i = \frac{1}{1 + e^{\mu + \alpha x_i}} \tag{1}$$

where x_i denotes the estimated weight of the plant just before the cold treatment. This formula yields a sigmoid curve (see below). The parameter α is the regression coefficient. In this case, α is always <0, because the flowering probability is positively correlated with plant size. The more negative α, the steeper the curve. The parameter μ determines the value of p_i if there is no influence of size. The smaller μ, the smaller the intercept of the curve with the *y*-axis. A large μ causes the curve to start increasing only at larger plant sizes, thereby shifting the curve to the right. Parameter μ is thus related to the population minimum size for flowering, although not directly, because the effect of μ depends on how large α is. Both parameters are estimated by maximization of the likelihood function $L(\mu,\alpha)$ defined by

$$L(\mu,\alpha) = \prod_{i=1}^{n} p_i^{q_i}(1-p_i)^{1-q_i} \tag{2}$$

where p_i denotes the estimated flowering probability for the *i*th plant with size x_i, calculated in formula (1), q_i is the actual stage of plant *i*: $q_i = 0$ for vegetative plants, $q_i = 1$ for flowering plants.

Differences among fitted curves for *k* groups can be tested by calculating the log (L_{max}) for both the pooled data and the groups separately. The test statistic Λ is defined by

$$\Lambda = 2\left[\sum_{j=1}^{k} [\log(L_{\max,j})] - \log(L_{\max,\mathrm{pooled}})\right] \tag{3}$$

Λ follows approximately a chi-square distribution with $2k-2$ degrees of freedom.

Table 2. Means and ranges of estimated plant dry weights (dw) for all populations, just before the cold treatment, and percentage flowering after the cold period. The transition range gives the estimated dry weights (g) of the smallest flowering plant (min fl) and the largest non-flowering plant (max veg) in each group. It indicates approximately the range of threshold sizes within a group. The values for Oulu are in parentheses because this population has no true transition range (see text). The last column gives the percentage of the total weight range covered by the transition range, a measure of variation in threshold size within a group

Population	*n*	Mean dw (g) ±SD	dw range (g)	% flowering	Transition range min fl	Transition range max veg	% of dw range
Meijendel	50	3·74±1·14	1·63–6·21	70	2·18	5·85	80
Holkham	48	3·03±1·36	0·99–6·43	15	2·85	5·84	55
Bordeaux	50	3·69±0·81	2·00–5·50	88	2·61	3·85	35
Oulu	50	2·85±0·91	1·11–4·70	78	(2·14	1·96	0)
Turku	50	3·73±0·89	2·25–5·70	54	3·47	4·07	17

Confidence intervals for μ and α are calculated from the covariance matrix of μ and α. The method does not allow a direct test for differences in μ or α separately.

RESULTS

Experiment 1: variation in cold requirement

None of the plants flowered in their first season, despite their large size, indicating that all plants had a vernalization requirement. All plants flowered in 1988, in their second year. The mean number of days until the opening of the first flower for each site (Table 1) showed no significant correlation with latitude (Spearman rank correlation: $\rho = 0{\cdot}28$; $n = 22$; $P > 0{\cdot}20$).

Experiment 2: variation in threshold size

Clear differences were found between the populations (Table 2). Flowering percentage for each group ranged from 15 to 88%. The five populations differed significantly in flowering percentage ($\chi^2 = 67{\cdot}774$; df = 4; $P < 0{\cdot}0001$). In spite of the common growing conditions, plants from Holkham and Oulu were on average significantly smaller just before the cold treatment than plants from Meijendel, Bordeaux, and Turku (ANOVA on estimated dry weight: $F = 8{\cdot}632$; df = 244, 4; $P < 0{\cdot}0001$). However, the flowering percentage was not correlated with the average dry weight (Spearman rank correlation: $\rho = -0{\cdot}1$; $n = 5$; $P = 0{\cdot}90$).

Figure 1 gives the fitted curves, based on the maximum likelihood analysis of the data of each population. Fitted curves follow the observed data closely. Meijendel and Holkham differ from Oulu, Turku, and Bordeaux, being less steep. The results of the maximum likelihood analysis are given in Table 3. The Oulu data differ from the other curve fits, due to a sudden switch from vegetative to flowering, which cannot properly be described by formula (1). This is the cause of the wide confidence intervals for both μ and α. All curves differ significantly from each other, when log likelihoods are compared (Table 4).

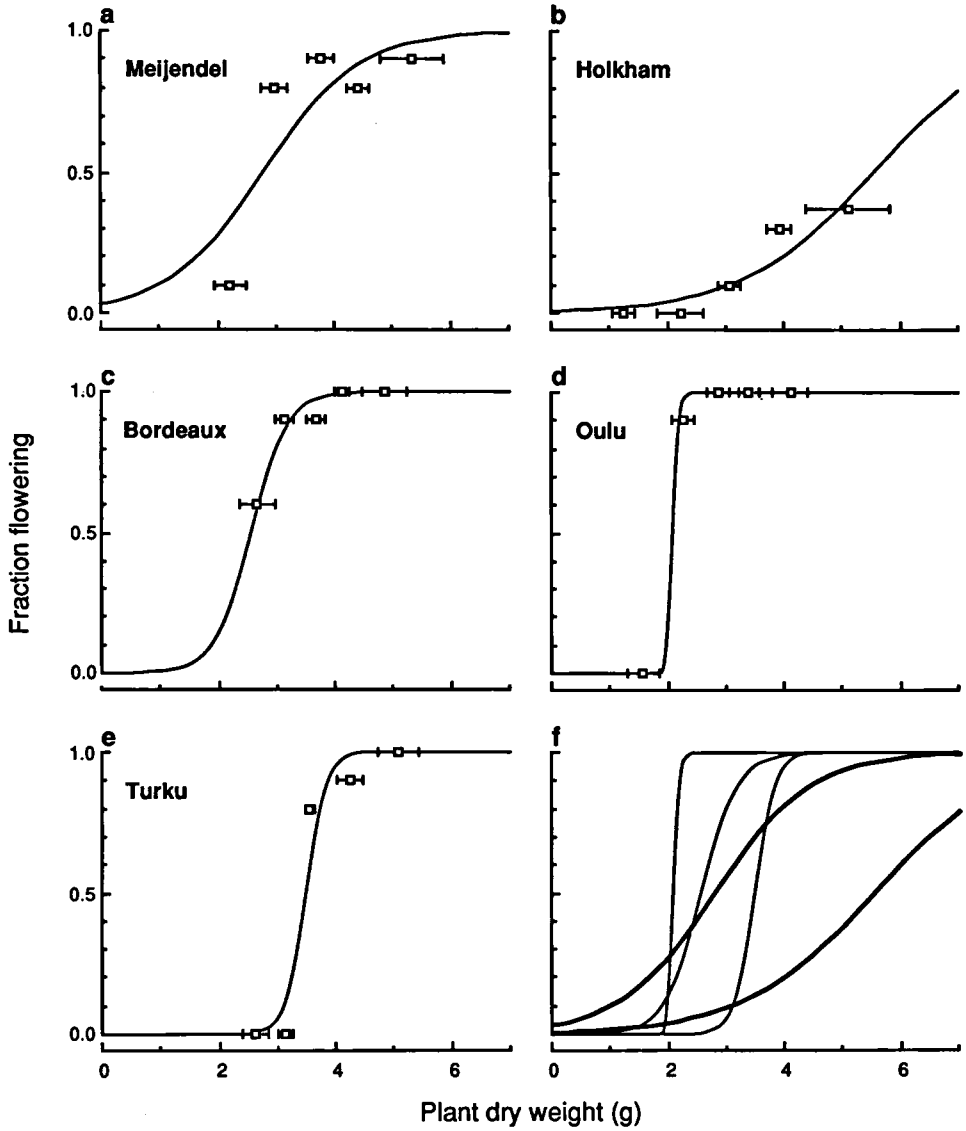

Fig. 1. Relationship between estimated plant dry weight before the cold treatment and the fraction flowering for each of the five populations (a–e) of *Cynoglossum officinale*. The squares indicate for groups of 10 plants (8 for the largest Holkham group), sorted by increasing plant size, the mean dry weight (±SD) and the fraction flowering in each group. The curves are the fitted maximum likelihood estimates for formula (1). In (f), all curves are shown together for comparison. Bold lines indicate the two natural populations, Meijendel and Holkham.

Within-population variation in threshold size. The transition range (Table 2) is a minimum estimate of the range in threshold sizes present in a group. The transition ranges for the two natural populations are wide, indicating the presence of a large variation in threshold sizes. For the three botanic gardens, however, this range is quite narrow, with the extreme case of Oulu (0%). Oulu is the only group with a sharp boundary between vegetative and flowering plants, without any overlap in size between each stage. The differences between the natural populations and the cultivated ones in within-population variation show even

Table 3. Results of the maximum likelihood analysis. Values of $\log(L_{max})$, means and 95% confidence intervals for the parameters μ and α, and the midpoint weight, the weight at which formula (1) predicts 50% flowering, are given (see Methods for a description of the analysis). For the pooled data, $\log(L_{max}) = -131{\cdot}0528$

Population	$\log(L_{max})$	Max. likelihood estimate		95% confidence intervals		Midpoint weight (g)
		μ	α	μ	α	
Meijendel	−23·7372	3·488	−1·253	2·68–4·30	−2·05–−0·45	2·78
Holkham	−16·3543	4·923	−0·891	3·81–6·03	−1·64–−0·15	5·53
Bordeaux	−11·6672	8·406	−3·280	6·09–10·73	−5·94–−0·62	2·56
Oulu	−0·0014	180·942	−88·093	−294·11–656·00	−683·67–507·49	2·05
Turku	−11·1975	21·574	−6·173	20·05–23·10	−10·35–−1·99	3·49

Table 4. The results of testing for differences in fitted curves between populations. Above the diagonal the values of Λ for all possible combinations of populations, below the diagonal the *P*-values from the chi-square distribution

	Population	Λ				
		Meijendel	Holkham	Bordeaux	Oulu	Turku
	Meijendel	—	26·928	7·226	36·475	17·685
	Holkham	0·0001	—	55·547	90·315	29·611
P	Bordeaux	0·0270	0·0001	—	21·025	27·930
	Oulu	0·0001	0·0001	0·0001	—	71·407
	Turku	0·0001	0·0001	0·0001	0·0001	—

more clearly in the fitted curves (Fig. 1). These show a gradually increasing flowering probability with plant size for both Meijendel and Holkham, and steep transitions for the botanic populations. This difference is caused by smaller (less negative) α's for Meijendel and Holkham (Table 3).

Differences among populations in 'average' threshold size. The 'average' threshold size is approximated by calculating from formula (1) the size at which the flowering probability is 0·5, the so-called midpoint weight (Table 3). Different populations have quite different midpoints. Holkham has a very high 'average' threshold size; the curve is so strongly shifted to the right that we did not find 100% flowering in the dry-weight range we worked with. Oulu has the lowest midpoint weight. Midpoint values were not correlated with the width of the transition range (Spearman rank correlation: $\rho = 0{\cdot}5$, $n = 5$, $P = 0{\cdot}50$).

DISCUSSION

None of the tested populations showed annual behaviour, so there seems to be no variation in presence or absence of vernalization requirement. Annual behaviour has been found in the congeneric *Cynoglossum creticum* Miller and *Cynoglossum hungaricum*

Simk. (De Jong & Klinkhamer 1989). In *C. creticum*, plants originating from lower latitudes also flowered earlier in the season.

There exist clear differences among and within populations in threshold size for flowering in *Cynoglossum officinale*. The plants originating from natural populations, Meijendel and Holkham, showed the largest within-population variation. The plants from botanic gardens showed much less variation. This could well be caused by genetic drift, because of the small population sizes in the botanic gardens. This can lead to such extreme cases as for Oulu, in which no variation in threshold size could be detected: all plants larger than *c.* 2·0 g flowered, and none below this weight did so.

It has been suggested that one finds a large overlap in size between vegetative and flowering plants because the actual factor regulating flower induction would not be plant size, but some other internal factor, only weakly correlated with plant size (De Jong *et al.* 1986). This hypothesis does not hold in the face of our results, because in that case we would expect equal within-population variation for all populations. For the same reason, Lacey's idea (1986) that good growing conditions for an individual plant can lead to a higher threshold size seems not applicable to *Cynoglossum officinale*. All in all, results strongly indicate the possibility that the differences found indeed have a genetic basis.

The data for Bordeaux, Oulu, and Turku suggest a history of selection in the botanic gardens for small threshold sizes. Cultivating monocarpic perennials in a botanic garden often involves collecting seed from a bed that flowers in its second year, and then turning and replanting the bed. This would constitute truncation selection for true biennials, causing both reduced variance and a shift to smaller threshold sizes. This selection force contrasts with predicted selection forces under field conditions from an optimization model of De Jong *et al.* (1989). This model predicts strong selection against threshold sizes smaller than the predicted optimum of 2 g, and weak selection on sizes larger than the optimum. The model was based on data from Meijendel. Fom this we would expect a sudden increase in flowering probability above 2 g, and a much more gradual approach to 100% flowering for Meijendel plants. Figure 1a shows that this description might fit the Meijendel data better than the symmetrical curve that is produced by formula (1).

Plants from Holkman flowered at a much higher weight than plants from Meijendel. Field measurements in both populations (unpublished data) confirmed these results. As yet, we have no data on size-dependent growth and survival from Holkham that might clarify the differences in threshold size between the two populations. The optimization model (De Jong *et al.* 1989) predicts larger threshold sizes when the variation in seedling recruitment or in reproductive effort is higher. The chances for further growth—and a higher seed production in the next year—for adult rosettes also influence optimal threshold size. As long as plants can still grow, delay of flowering is profitable. Better conditions for further rosette growth in Holkham could have caused the difference. These conditions might be, for instance, the absence of herbivores with a preference for larger plants (Prins *et al.* 1992).

For natural selection to occur, three conditions have to be met: phenotypic variation, fitness differences, and a genetic basis to the character studied (Endler 1986). This paper shows the presence of variation within populations. Optimization models like the ones of Kachi & Hirose (1985) and De Jong *et al.* (1989) give strong indications of fitness differences for different threshold sizes. Artificial-selection experiments are being carried out at the moment to quantify the heritability of threshold size for flowering. Results up to now indicate the possibility that natural selection acts upon threshold size for flowering in *Cynoglossum officinale*.

ACKNOWLEDGEMENTS

We thank E. Meelis for assistance in the mathematical analysis of the data, and E. van der Meijden for his useful comments. This is a publication of the 'Meijendel-comité', new series no. 105.

REFERENCES

Augspurger, C.K. (1985): Demography and life history variation of *Puya dasylirioides*, a long-lived rosette in tropical subalpine bogs. *Oikos* **45:** 341–352.

Baskin, J.M. & Baskin C.C. (1979a): Studies on the autecology and population biology of the monocarpic perennial *Grindela lanceolata*. *Am. Midl. Nat.* **102:** 290–299.

Baskin, J.M. & Baskin, C.C. (1979b): Studies on the autecology and population biology of the weedy monocarpic perennial, *Pastinaca sativa*. *J. Ecol.* **67:** 601–610.

Boorman, L.A. & Fuller, R.M. (1984): The comparative ecology of two sand dune biennials: *Lactuca virosa* L. and *Cynoglossum officinale* L. *New Phytol.* **69:** 609–629.

De Jong, T.J. & Klinkhamer, P.G.L. (1989): *Cynoglossum*. In: Halevy, A.H. (ed.): *CRC Handbook of Flowering*. 281–285. CRC Press, Boca Raton, Florida, USA.

De Jong, T.J., Klinkhamer, P.G.L. & Boorman, L.A. (1990): Biological flora of the British Isles: *Cynoglossum officinale* L. *J. Ecol.* **78:** 1123–1144.

De Jong, T.J., Klinkhamer, P.G.L. & Prins, A.H. (1986): Flowering behaviour of the monocarpic perennial *Cynoglossum officinale* L. *New Phytol.* **103:** 219–229.

De Jong, T.J., Klinkhamer, P.G.L., Geritz, S.A.H. & Van der Meijden, E. (1989): Why biennials delay flowering: an optimization model and field data on *Cirsium vulgare* and *Cynoglossum officinale*. *Acta Bot. Neerl.* **38:** 41–55.

Endler, J.A. (1986): *Natural Selection in the Wild*. Monographs in Population Biology 21, Princeton University Press, Princeton, New Jersey, USA.

Gross, K.L. (1981): Predictions of fate from rosette size in four 'biennial' plant species: *Verbascum thapsus, Oenothera biennis, Daucus carota*, and *Tragopogon dubius*. *Oecologia* **48:** 209–213.

Gross, K.L. & Werner, P.A. (1983): Probabilities of survival and reproduction relative to rosette size in the common burdock (*Arctium minus*: Compositae). *Am. Midl. Nat.* **109:** 185–193.

Hirose, T. & Kachi, N. (1982): Critical plant size for flowering in biennials with special reference to their distribution in a sand dune system. *Oecologia* **55:** 281–284.

Kachi, N. (1990): Evolution of size-dependent reproduction in biennial plants: a demographic approach. In: Kawano, S. (ed.): *Biological Approaches and Evolutionary Trends in Plants*. 367–385. Academic Press, London.

Kachi, N. & Hirose, T. (1985): Population dynamics of *Oenothera glazioviana* in a sand-dune system with special reference to the adaptive significance of size-dependent reproduction. *J. Ecol.* **73:** 887–901.

Klinkhamer, P.G.L. & De Jong, T.J. (1983): Is it profitable for biennials to live longer than two years? *Ecol. Model.* **20:** 223–232.

Klinkhamer, P.G.L. & De Jong, T.J. (1987): Plant size and seed production in the monocarpic perennial *Cynoglossum officinale* L. *New Phytol.* **106:** 773–783.

Klinkhamer, P.G.L. & De Jong, T.J. (1993): Biological flora of the British Isles: *Cirsium vulgare* (Savi) Ten. *J. Ecol.* **81**(1): 177–192.

Klinkhamer, P.G.L., De Jong, T.J. & Meelis, E. (1987a): Delay of flowering in the 'biennial' *Cirsium vulgare*: size effects and devernalization. *Oikos* **49:** 303–308.

Klinkhamer, P.G.L., De Jong, T.J. & Meelis, E. (1987b): Life-history variation and the control of flowering in short-lived monocarps. *Oikos* **49:** 309–314.

Klinkhamer, P.G.L., De Jong, T.J. & Meelis, E. (1991): The control of flowering in the monocarpic perennial *Carlina vulgaris*. *Oikos* **61:** 88–95.

Lacey, E.P. (1986): The genetic and environmental control of reproductive timing in a short-lived monocarpic species *Daucus carota* (Umbelliferae). *J. Ecol.* **74:** 73–86.

Lacey, E.P. (1988): Latitudinal variation in reproductive timing of a short-lived monocarp, *Daucus carota* (Apiaceae). *Ecology* **69:** 220–232.

Lee, J.M. & Hamrick, J.L. (1983): Demography of two natural populations of musk thistle (*Carduus nutans*). *J. Ecol.* **71:** 923–936.

Primack, R.B. & Kang, H. (1989): Measuring fitness and natural selection in wild plant populations. *Annu. Rev. Ecol. Syst.* **20:** 367–396.

Prins, A.H., Nell, H.W. & Klinkhamer, P.G.L. (1992): Size-dependent herbivory on *Cynoglossum officinale* L. *Oikos* **65:** 409–413.

Prins, A.H., Vrieling, K., Klinkhamer, P.G.L. & De Jong, T.J. (1990): Flowering behaviour of *Senecio*

jacobaea: effects of nutrient availability and size-dependent vernalization. *Oikos* **59:** 248–252.

Reinartz, J.A. (1984): Life history variation of common mullein (*Verbascum thapsus*) I. Latitudinal differences in population dynamics and timing of reproduction. *J. Ecol.* **72:** 897–912.

Reznick, D.A., Bryga, H. & Endler, J.A. (1990): Experimentally induced life-history evolution in a natural population. *Nature* **346:** 357–359.

Stearns, S.C. (1976): Life-history tactics: a review of the ideas. *Q. Rev. Biol.* **51:** 3–47.

Van der Meijden, E. & Van der Waals-Kooi, R.E. (1979): The population ecology of *Senecio jacobaea* in a sand dune system. I. Reproductive strategy and the biennial habit. *J. Ecol.* **67:** 131–153.

Van der Meijden, E., Klinkhamer, P.G.L., De Jong, T.J. & Van Wijk, C.A.M. (1992): Meta-population dynamics of biennial plants: how to exploit temporary habitats. *Acta Bot. Neerl.* **41:** 249–270.

Werner, P.A. (1975): Predictions of fate from rosette size in teasel (*Dipsacus fullonum* L.). *Oecologia* **20:** 197–201.