

SELECTIVE SEED ABORTION INCREASES OFFSPRING SURVIVAL IN *CYNOGLOSSUM OFFICINALE* (BORAGINACEAE)¹

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Selective embryo abortion is one of the evolutionary explanations for the surplus of ovules found in many plant species. To manipulate the level of embryo abortion, we removed ovules and applied nutrients to plants of *Cynoglossum officinale* (Boraginaceae) after they started to flower. From these two treatments and a control series, seeds were collected, germinated, and transplanted in the field to assess the quality of the resulting offspring. Nutrient addition did not increase the number of seeds per flower significantly. Fewer embryos were aborted in the ovule removal treatment. The seeds produced in the ovule removal treatment had a significantly greater mass and significantly lower survival than the offspring from the control group. This difference in survival indicates that offspring of lower quality are selectively aborted in the control group. Offspring from the nutrient addition treatment survived longer. The offspring of the treatments did not differ significantly from the control group in growth. Simple mathematical calculations, based on the differences in offspring quality that we found, indicate that the selective abortion hypothesis can be an important factor explaining the advantage of the “surplus production” of ovules.

Key words: bet-hedging strategy; Boraginaceae; *Cynoglossum officinale*; embryo abortion; offspring quality; seed abortion.

Many plant species produce far more ovules than seem necessary for the production of their seeds. In many cases a low seed-to-ovule ratio is not caused by a lack of compatible pollen (Willson and Burley, 1983). The review of Burd (1994) demonstrated that, although pollen might be limited in several cases, percentage seed set is only slightly increased when conditions of pollen limitation are relaxed. Three nonexclusive evolutionary hypotheses have been proposed (Stephenson, 1981) for this “surplus of flowers”: (1) An exploitation of extremely favorable conditions, or an ovary reserve in case of loss of ovaries due to unpredictable mortality have been lumped together under the concept of “bet hedging” (e.g., Stephenson, 1980; Udovic, 1981; Kozłowski and Stearns, 1989; Ehrlén, 1991). (2) Flowers that act only as pollen donors serve to increase male fitness (e.g., Willson and Price, 1977; Queller, 1983; Sutherland and Delph, 1984). (3) Selective abortion of offspring of relatively low quality can free resources for higher quality offspring and thereby increase the fitness of the maternal plant (e.g., Darwin, 1883; Janzen, 1977; Stephenson, 1981; Marshall and Ellstrand, 1988; Marshall and Folsom, 1991; Melser, Rademaker, and Klinkhamer, 1997). This effect is expected to be most distinct when resources for producing seeds are limited.

To test this last hypothesis it has to be shown that: (1) otherwise viable embryos are aborted and (2) offspring quality increases with the level of abortion. If the removal of flowers or ovules increases seed set in the remaining flowers and ovules, then the proof that aborted embryos were potentially viable is established. In a fruit thinning experiment by Lee and Bazzaz (1986) with *Cassia fasciculata*, one randomly selected fruit in every other inflorescence was allowed to mature, while

the other fruits were removed. The proportion of ovules producing mature fruits was significantly higher in treatment plants in comparison to the control plants. When fruits were removed from the upper branches of *Agave mckelveyana*, the lower branches showed a compensation in fruit production (Sutherland, 1987). The artificial removal of half of the fruits on inflorescences decreased the abortion level in the remaining fruits of *Lotus corniculatus* (Stephenson and Winsor, 1986). Besides the increase in seed number per flower of the hand-thinned inflorescences, the offspring from the hand-thinning treatment grew more slowly and produced fewer flowers, inflorescences, and fruits compared to the control group (Stephenson and Winsor, 1986). Ovules that normally would have been aborted could develop into seeds after removal of other ovules in the nutlets of *Cryptantha flava* (Casper, 1983, 1984, 1988). Seeds produced in the ovule-removal treatment showed a reduced percentage germination (Casper, 1988). In *Phaseolus coccineus*, the destruction of some ovules in the ovary increased the probability that the remaining ovules would produce a mature seed. However, compared to seeds from control fruits, the progeny from the experimental fruits were less vigorous (Rocha and Stephenson, 1991).

Evidence against the hypothesis of abortion of viable embryos was presented by two of Andersson's experiments (1990, 1993). With the removal of half of the ovules in *Anchusa officinalis*, the seed set in the remaining ovules was equal to the seed set in the ovules of the control group (Andersson, 1990). When half of the flowers in the flower heads were removed in *Achillea ptarmica*, the mean ratio of seed per ovule was not increased in comparison to the control flower heads (Andersson, 1993). Plants were apparently not forced by the flower removal to produce seeds that otherwise would have been aborted for these two species. Clearly, the results of the fruit or ovule removal experiments are not unequivocal. We anticipate that the largest effect of fruit or ovule removal experiments will be achieved if treatments are applied to whole plants. If different treatments are applied within one plant, these results will not be different from the application

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of a treatment to a whole plant, only if allocation of resources is restricted between flowers. An effect on seed set per flower might be masked if resources can be reallocated between different flowers of the same plant. Viable embryos can still be aborted after hand thinning if species store their resources for another flowering season rather than reallocating them to embryos of lower quality in the actual flowering season. This does not necessarily exclude an effect, but may account for the fact that no effect is found. To prevent storage for another flowering season, monocarpic species can be used.

Here we study offspring quality in relation to embryo abortion by applying treatments to whole plants in *Cynoglossum officinale* (L.), a monocarpic perennial of calcareous grasslands and vegetated dunes. The experiment addressed the following questions: (1) Are otherwise viable embryos aborted naturally? and (2) Do increased abortion levels lead to offspring with a higher average quality? In other words, do retained offspring survive better or grow faster than otherwise aborted offspring?

MATERIALS AND METHODS

Species—*Cynoglossum officinale* (L.) is a member of the Boraginaceae. This monocarpic species is a rosette forming, self-compatible perennial. From the main flowering stem, cymes (side branches) diverge on which flowers develop sequentially. Every day, one to two new flowers (size ~1 cm) open at each cyme. Every flower remains open for ~2 d. Numbers in the field range from 80 to >300 flowers per plant. The dull red-purple corolla fades to blue before abscission. The most common flower visitors in our study area are bumble bees, and honey bees are less common pollinators (de Jong and Klinkhamer, 1989). Flowers are hermaphroditic with five anthers and four ovules. The ovules are symmetrically arranged in a square and may develop into four seeds. Abortion of seeds occurs in all stages of development, but mostly in the early stages (C. Melsner, personal observation). Usually the smaller nutlets are aborted seeds. However, sometimes the seed coat of an aborted seed has the average size of a ripe seed, but no embryo is present and abortion is indicated by a considerably lower mass of the seed. Although in the field the average seed set of *C. officinale* is only one seed per flower (i.e., per four ovules), natural populations in our study area are not pollen limited (de Jong and Klinkhamer, 1989; Melsner et al., unpublished data). Frequency distributions of number of seeds per flower are given in de Jong and Klinkhamer (1989). Seeds do not remain attached to each other after ripening and are dispersed individually.

Pollination—Plants were collected from a natural population of *C. officinale* in the dunes of Meyendel, near The Hague, The Netherlands. Thirty individual plants of approximately equal size were collected in April 1997 as rosettes initiating elongating of a flowering stem. Each plant was randomly assigned to one of three treatments. To exclude any resource allocation between treated flowers and control flowers a single treatment to a whole plant was applied. After collection, the plants were grown in 3-L pots (except treatment 2) with a mixture of sand and soil (50/50%) and placed in an experimental garden and permitted to be openly pollinated. Plants were placed to avoid contact between adjacent individuals.

In order to manipulate embryo abortion levels, ten plants were used for each of the three treatments, and each plant received one treatment: (1) ovule removal: 4–5 d after pollination, prior to visible ovule development, three of the four ovules in each flower on the whole plant were carefully destroyed and removed with a needle. The remaining ovule was left visibly undamaged by the needle; (2) nutrient addition: extra space (10-L pot) and nutrients (100 mL of a nutrient solution, i.e., macronutrients according to Steiner [1968], micronutrients slightly adapted from Arnon and Hoagland [1940] three times a week) were provided, and; (3) control plants without ovule removal or nutrient addition.

With ovule removal, we simulated herbivory or other physical damage to

the ovules. With adding nutrients to the environment, we created unpredictable favorable environmental conditions in terms of resources. In addition to open pollination, hand pollination was applied with a small brush to all open flowers. The brush distributed adhering pollen among all flowers treated. The pollination was carried out on all open flowers, three times a week, to ensure sufficient pollination. Hand pollination is known to supply viable pollen, as relatively high seed numbers per flower (2.77–2.86) were achieved after hand pollination (Luckerhoff, unpublished data; Melsner, unpublished data). Flowers were marked with a drop of paint to identify the date of opening. The treatments were applied during the entire flowering period, which lasted from 12 May until 27 June 1997.

The proportion of ovules that produced mature seeds was calculated for each plant. The average number of seeds per flower per plant, the average seed mass per plant, the total number of flowers, and the total number of seeds per plant were determined and analyzed by analysis of variance with treatment as a fixed variable. The two experimental groups were compared to the control group with contrast statements (Sokal and Rohlf, 1995).

Offspring performance—To determine the quality of the offspring, we randomly selected 60 seeds per maternal plant (1800 seeds in total), but due to limited seed production in two maternal plants we only selected a total of 1755 seeds. The seeds were weighed and the seed coat was carefully sliced at the top to break dormancy. Empty seed coats were excluded from the rest of the experiment, leaving 1569 seeds for germination on wet filter paper. Each seed was placed into a separate cell of a small tray, watered for germination, and then placed into a growth chamber. Day and night temperature in the growth chambers were, respectively, 20°C and 15°C, and relative humidity ranged between 60 and 85%. After germination the seedlings were transplanted into 9 cm diameter pots. The plants were transplanted into the field at Meyendel, in early March 1998, 35 d after the start of germination. The habitat was a small valley in vegetated sand dunes. The area was fenced to exclude large herbivores. All plants were at least 20 cm apart from each other. The plants were watered regularly during the first 3 wk to reduce mortality due to the transfer. Initially survival was censused weekly. The frequency decreased to once every 3 wk in October and November of 1998. During the winter no census could take place, because *C. officinale* loses its aboveground leaves during the cold period. From April 1999 the census was restarted and continued once every 2 wk until the end of the experiment in October 1999, when only 12 plants were still alive. No plants from our experiment flowered in 1999. The number of leaves and the length of the longest leaf of the surviving plants were determined in March 1998 before transplantation in the field, and after transplantation in May 1998, September 1998, and July 1999.

A survival analysis was performed with Cox's model (Lagakos, 1992) in SPSS (Statistical Package for the Social Sciences). With this model, we compared the survival functions of two groups with a log-rank test with multiple explanatory variables. The survival function is defined as the proportion of individuals who survive beyond a certain time. To detect whether seed mass has an effect on survival, the regression between seed mass and survival for the different treatments was analyzed. Seed mass was included as a continuous covariate, as it met the assumption of a proportional hazard function. The hazard function is defined as the negative of the slope of the survival curve when the latter is plotted on a logarithmic scale. Treatments were included as categorical covariates. The hazard functions of ovule removal (treatment 1) and addition of nutrients (treatment 2) were compared with the hazard function of the control group (treatment 3). In addition, the number of surviving individuals per treatment 10 d after the majority of the seeds had germinated was analyzed with a *G* test for proportionality (Sokal and Rohlf, 1995) without a correction for mass of the individual seeds. An index for leaf area was calculated as the number of leaves per plant times length of the longest leaf. This leaf area index is highly correlated with whole-plant dry mass (Wesseling et al., 1993). Differences in leaf area index between treatments were analyzed by ANOVA, with seed mass and germination day as covariates. The two experimental treatments were compared to the control group with a contrast statement.

TABLE 1. ANOVA results for seeds per ovule, seed mass, total number of flowers, and total number of seeds for the plant groups control, nutrient addition, and ovule removal. For each group $N = 10$.

Variable	Nutrient addition		Control		Ovule removal		MS	F	df	P
	Mean	SE	Mean	SE	Mean	SE				
Seeds per ovule	0.27	0.033	0.21	0.022	0.49***	0.031	0.216	25.43	2,27	<0.0001
Seed mass (g)	0.020	0.0003	0.019	0.0007	0.025***	0.0011	0.0001	11.84	2,27	0.0002
No. of flowers	306.8**	39.6	174.1	17.8	173.6	12.7	58919	8.64	2,27	0.0013
No. of seeds	343.1*	76.1	149.9	24.3	83.8*	7.77	181553	8.45	2,27	0.0014

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, contrast between mean of treatment and mean of control.

RESULTS

Mother plants—The ovule removal treatment had a highly significant effect on the number of seeds per ovule (Table 1; Fig. 1). The control group produced on average 0.21 seeds per ovule (range 0.08–0.29), and with the removal of three of the four ovules, the seed set in the remaining ovule was more than doubled compared to the control group (mean = 0.49; range 0.31–0.63). This increase in the number of seeds per ovule after ovule removal revealed that under natural conditions on average at least 56.8% of the viable embryos were aborted in the control group. Although the number of seeds per ovule increased by a mean of 28% with the addition of nutrients (mean = 0.27; range 0.16–0.48), this was insignificant.

The overall mean value of seed mass was 0.0226 g. After ovule removal the seed mass significantly increased in comparison to the control by 33% [(0.0248 – 0.0186)/0.0186] (Table 1). Seed mass of the nutrient addition treatment did not differ significantly from the control.

The average number of flowers per plant increased significantly (76%) in the nutrient addition treatment, but the ovule removal treatment did not differ from the control group (Table 1).

The total number of seeds produced per plant was significantly reduced by ovule removal (83.8 ± 7.77) and increased by nutrient addition (343.1 ± 76.1) relative to the control

treatment (149.9 ± 24.3 ; Table 1). The nutrient addition treatment more than doubled the number of seeds produced compared to the control group. This doubling was a consequence of higher flower production rather than an increase in seed number per flower. Although ovule removal increased the number of seeds per ovule, the total number of seeds per plant significantly decreased by 44% as compared to the control group. The decrease in the total number of seeds per plant is due to the decrease in available ovules per plant, induced by the treatment itself.

Offspring survival—Seeds with higher mass produced seedlings that lived longer (Fig. 2), although the shape of this relationship is not necessarily linear. For the sake of clarity in the graph, seeds are presented in four mass classes. However, all analyses are performed with individual seed masses. Because seed mass was a significant factor in the survival analysis ($P < 0.0001$), it is included as a continuous covariate. Twelve plants that are included in Fig. 2 were still alive at the time of analysis (October 1999), when the final census was done (573 d).

Offspring survival rate is calculated with and without correcting for seed mass. First we present a survival analysis with a correction for seed mass by including seed mass as a covariate. Offspring from both experimental treatments differed in survival from the control group (Fig. 3; $P < 0.0001$). After removal of ovules, the offspring from the remaining ovules had a lower survival than the control group ($P < 0.0001$). The offspring from the nutrient treatment survived slightly but significantly longer than the offspring from the control group ($P = 0.0004$). Mortality was high during germination (after 6–8

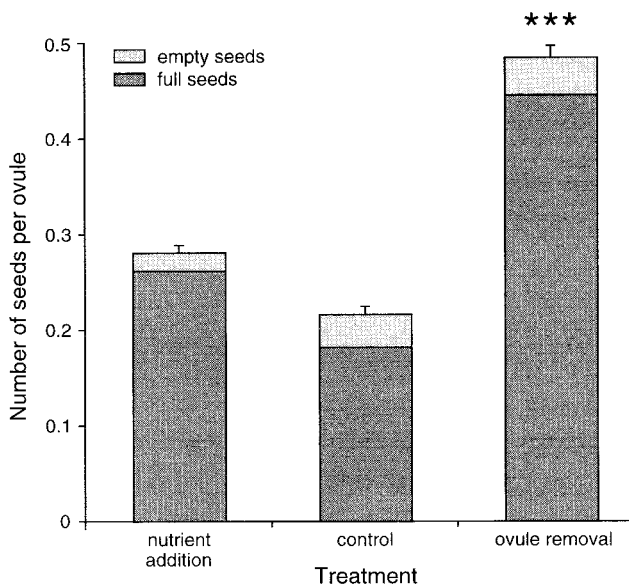


Fig. 1. The average number of seeds per ovule (+ SE) for three groups for both empty and full seeds. For results of the analysis of variance see text (*** $P < 0.001$, contrast between treatment and control).

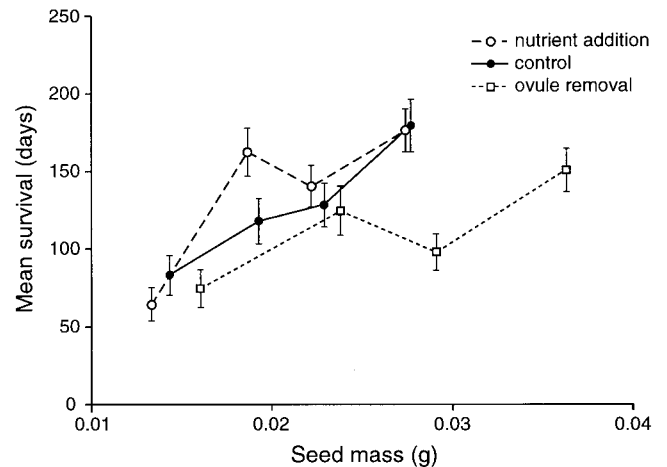


Fig. 2. The relation between survival and seed mass for three groups. Seed mass is categorized in four groups with equal numbers per treatment.

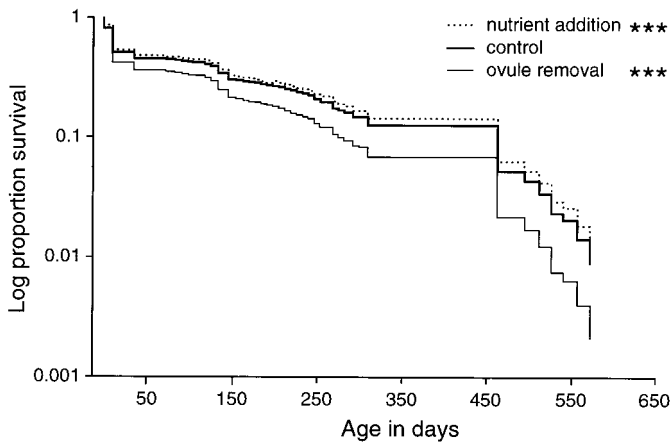


Fig. 3. Survival function of percentage surviving offspring (log scale) for three groups. The covariate seed mass is for all groups set to the overall mean value (0.0226 g), (***) $P < 0.001$, contrast between treatment and control.

d) and the 10 d thereafter when the plants were transferred to pots. The percentage of surviving individuals after 10 d (corrected for seed mass as a covariate) was 53% in the control group, 55% for the nutrient addition treatment, and 43% for the ovule removal treatment (Fig. 3). Second, to determine differences in survival without correcting for seed mass, we calculated the percentages of offspring surviving from each group after 10 d. Of the 486 individuals of the control group, 249 (51.2%) survived 10 d; of the 558 individuals of the nutrient treatment, 302 (54.1%) survived; of the 525 individuals of the ovule removal treatment, 230 (43.8%) survived. This gives a difference in survivorship between the control plants and the plants of the ovule removal of 14.4% [(51.2 - 43.8)/51.2]. The G test for proportionality on these data (thus without correction for seed mass) yields a significant difference in proportions among the three groups 10 d after germination ($X^2 = 5.991$; $df = 2$; $P = 0.050$).

Offspring size—Initially, the covariates seed mass and germination day were significantly affecting leaf area in March, 1998 (seed mass $P < 0.0001$; germination day $P = 0.0041$), but their effects disappeared from the second census onwards (May, 1998). Leaf area (as an index of plant growth) of the offspring was unaffected by the treatments on all dates (in March, May, and September 1998, and July 1999 the P values were 0.505, 0.807, 0.249, and 0.073, respectively).

DISCUSSION

Embryo abortion—The removal of three of the four ovules in each flower increased the average seed number per ovule; we can, therefore, conclude that otherwise viable embryos are aborted in *C. officinale*. The increase in number of seeds per ovule in the ovule removal treatment furthermore shows that pollen was not limiting for seed production in the control group. In contrast to our findings, the removal of half of the ovules in *Anchusa officinalis* or removal of half of the flowers in the flower heads in *Achillea ptarmica* did not increase the mean ratio of seed per ovule (Andersson, 1990, 1993). However, it is possible that part of the negative results is caused by the design of these experiments. Firstly, both *A. officinalis* and *A. ptarmica* from Andersson's experiments (1990, 1993) are perennials. Viable embryos can still be aborted after hand

thinning if species store their resources for another flowering season rather than reallocating them to embryos of lower quality in the actual flowering season. This does not necessarily exclude an effect (see Casper, 1983; 1984 and 1988 for positive effects with the perennial *Crypthantha flava*), but may account for the fact that no effect is found. Secondly, in nearly all the experiments mentioned where flowers or ovules have been removed (Casper, 1983, 1984, 1988; Stephenson and Winsor, 1986; Andersson, 1990, 1993), the removal treatments were applied together with the control treatment within one plant (but see Lee and Bazzaz, 1986; Sutherland, 1987). The effect of flower or ovule removal can be masked or at least underestimated with reallocation of resources either to another flowering season or to the control flowers or inflorescences. Only if allocation of resources is restricted between flowers, the results of those experiments will not differ from an experimental design where a removal treatment is applied to all flowers of a whole plant. However, the integrated physiological unit of a plant, which acts relatively autonomous with respect to the assimilation, distribution, and utilization of carbon, varies greatly among species (Westoby and Rice, 1981; Watson and Casper, 1984). No information is present on restricted resource allocation between inflorescences of *A. officinalis* and *A. ptarmica* in the experiments of Andersson (1990, 1993). Moreover, the lower seed weights in fruits where ovules had been removed in *A. officinalis* suggest that resources are allocated between control fruits and experimental fruits of the same plant (Andersson, 1990). This may also account for the absence of an effect of flower or ovule removal on seed set per ovule.

Plants with all ovules intact that would have the same seed-to-ovule ratio as the plants from the ovule removal treatment would produce 1.97 seeds per flower. This would still represent only 49.3% of the maximum of four seeds and might indicate that not all natural pollinations result in viable embryos. It might also indicate that even in the ovule removal treatment many embryos are aborted. The latter may have obscured part of the potential effect of selective embryo abortion on offspring quality.

Species that have a fixed production of one seed per flower might have a selective advantage in dispersal of the seed units (Casper and Wiens, 1981). The seeds within a flower of *C. officinale*, however, do not remain attached to each other after maturing and are dispersed individually. Moreover, the seed set per flower in *C. officinale* is not fixed to one, but varies greatly from zero to the maximum of four seeds per flower. Mass per seed is not influenced by the number of seeds per flower where the individual seed is produced (*C. Melser*, unpublished data). Those features make it unlikely that the abortion of seeds in *C. officinale* is related to the dispersal of seeds in fixed units.

Bet-hedging theory explains the surplus production of ovules as a compensation for unexpected losses of ovules by herbivory, other physical damage, or as a possible means of exploitation of unpredictable environments. We simulated both conditions by removing ovules and by adding nutrients. However, seed set was still far from 100% and was not significantly increased with the addition of nutrients. Although the ovule removal treatment increased the average number of seeds per ovule considerably, seed set per ovule for *C. officinale* from the Meijendel population in the Netherlands never exceeded two seeds per flower under natural conditions in the field (e.g., Klinkhamer and de Jong, 1987; de Jong and Klinkhamer,

1989; de Jong, Klinkhamer, and Boorman, 1990). In growth chamber experiments, seed set can increase to 2.86 (SE = 0.17; $n = 4$; L. Luckerhoff, unpublished data); when flowers are removed, the remaining flowers on the plant received a gibberellin treatment, and the plants were cultivated at 15°C. The bet-hedging theory thus does not seem to be the sole explanation for the surplus production of ovules.

Offspring quality—To determine the quality of the resulting offspring, vigor and reproduction should be considered as well as survival. We did not examine reproduction of the offspring, but we assumed that vegetative growth gives a reliable indication of offspring performance. However, differences in offspring quality that are not expressed in vegetative life stages might come into expression at the reproductive stage (Melser, Bijleveld, and Klinkhamer, 1999). Preliminary results of a follow-up study suggest that *C. officinale* does not show differences due to selective abortion in reproductive success for male (pollen production and siring seeds) or female (total seed production) function (C. Melser and P. G. L. Klinkhamer, unpublished data).

Offspring survival—Random abortion of seeds by hand thinning leads to a lower survival of the offspring compared to abortion mediated by the maternal plant. The main differences in survival between the treatments and the control group originate during the first 10 d after germination. The initial differences are not counteracted by differences in survival during later life stages. The higher seed mass in the ovule removal treatment may have obscured some of the effects of selective embryo abortion. However, the test performed without correcting for seed mass revealed a significant difference in survival after 10 d among the three treatment groups.

The slightly higher survival rate of the seeds from the nutrient treatment relative to the seeds from the control series is partly due to the higher mass of the seeds from the nutrient treatment. Accordingly, we corrected for seed mass by including it as a covariate. We used a linear relationship between seed mass and longevity of the seedlings. Correcting for seed mass in this way, there was a positive effect of nutrient addition on seedling survival. This conclusion should be taken with caution, however, because our data do not exclude the possibility that the relation between seed mass and seedling survival is nonlinear.

All flowers were hand pollinated in addition to the open pollination. Relatively high pollen loads can increase the average quality of the offspring (Björkman, 1995; Quesada, Winsor, and Stephenson, 1996; Niesenbaum, 1999; but see Snow, 1990). Here, extra pollen was applied on all flowers of each experimental group and therefore is not likely to contribute to differences in offspring quality among the experimental groups. Moreover, additional hand pollination does not affect seed set under natural conditions (de Jong and Klinkhamer, 1989).

Offspring size—Apart from the increase in seed mass in the ovule removal treatment, there were no additional differences in offspring growth between the treatments and the control group. This result is rather surprising because we expected that differences in offspring quality would show in terms of both survival and growth. It should be kept in mind, however, that survival differences were strongest during the first 10 d after germination. Shortly after germination and subsequent plant-

ing of seedlings it is difficult to detect differences in growth rate because of the subtle effects of time required by seedlings to settle in the pots. Growth differences 10 d after germination, therefore, might be difficult to detect.

Possible causes of differences in quality—The removal of ovules decreased the survival of the emerging seeds. It could be argued that the treatment itself damaged the seed and, therefore, resulted in offspring of lower quality. However, the seeds had a higher mass than seeds from the control group, and the only remaining ovule in the ovule removal treatment had a higher chance of producing a seed. This is contrary to expectations if the treatment had had an adverse effect on seed maturing. However, by prematurely removing reproductive structures, one might upset initial source-sink relationships and thus affect plant resource levels (Casper, 1988) or induce chemical changes that divert resources from the developing seeds. In addition, forcing a flower to distribute resources to an ovule that it would not normally have matured might itself result in an inferior seed (Casper, 1988). In order to study selective embryo abortion, one should design an experimental set-up that does not involve the destruction of plant tissues. As Levri (1998) points out, differences in seed numbers per flower along the flowering season in the absence of pollen limitation could be used to study different levels of embryo abortion and their effects on offspring quality.

Low offspring quality in many species can be explained by inbreeding depression (e.g., Maynard Smith, 1978; Lloyd, 1980; Charlesworth and Charlesworth, 1987). Seed abortion might then reflect inbreeding depression at an early stage. The plants from the ovule removal treatment and the control group produced equal numbers of flowers. It is likely that with open pollination they have an equal percentage of selfed seeds (Vrieling et al., 1999). If there were differences in selfing rates, then the larger plants from the nutrient treatment are expected to have a higher percentage of selfing (Vrieling et al., 1999). Moreover, *C. officinale* is not subject to high levels of inbreeding depression (C. Melser and P. G. L. Klinkhamer, unpublished data). Selfing and outcrossing produce, on average, an equal number of seeds. Although the resulting offspring show slight differences in survival, both types of pollinations result in offspring of roughly equal reproductive performance (C. Melser and P. G. L. Klinkhamer, unpublished data).

Mortality of offspring was relatively high during germination and in the first 10 d thereafter. This can partly be explained by the occurrence of infection by fungi. We have, however, no reason to assume that differences in infection rate caused the differences in offspring survival between the treatment results. The germinating seeds each received a separate cell in a tray. Different mother plants, and thus different treatment groups, were systematically divided among the trays. Moreover, the overall mortality rates seemed to be within the range of those observed under natural field conditions. In a stable population with an individual seed production of 200 seeds per plant (Klinkhamer and de Jong, 1993), survival rate through to the reproductive stage was only 0.5%. Our experiment started with 1569 seeds, of which after 1.5 yr 12 plants (0.76%) survived.

Abortion and fitness—We find that the lower abortion level in the ovule-removal treatment led to a decrease in offspring fitness by 14.4% (data from 10 d after germination). What does this tell us about the possible importance of selective

abortion for the evolution of low seed-to-flower ratios in *C. officinale*? To address this question, we calculated for plants the minimum relative quality of their seeds required to produce an equal number of surviving offspring (= female fitness) for all plants. Plants with a lower seed number per flower require seeds of a relatively high quality to produce an equal amount of offspring in the F1 generation in comparison to plants with a higher seed number per flower. We assumed that the total reproductive budget for plants was fixed and that they can freely distribute resources among flowers and seeds with the constraint of a maximum seed-to-flower ratio of four. In *C. officinale* the costs of producing a seed are three times higher relative to the costs of producing a flower (data from Klinkhamer and de Jong, 1987, cited in Rademaker and de Jong 2000). We used two trade-offs for seed-to-flower ratio of two and three. If the trade-off and the total budget are known, we can calculate for all possible seed-to-flower ratios the corresponding number of seeds and flowers produced (Fig. 4A). The relative fitness of seeds from plants with 0.9 seeds per flower was set as the standard (this equals the mean seed set per flower from the control group). To produce an equal total female fitness we can then ask what relative fitness should seeds have if they come from plants with a different seed-to-flower ratio. This total female fitness isocline is plotted in Fig. 4B for two possible trade-offs between seeds and flowers and assuming the total budget to be 450 units with the costs of producing a flower set to one. The results show that, within a very broad range (from 4 to ~ 0.6 seeds per flower), large differences in the seed-to-flower ratio are accompanied by relatively small differences in total seed production (Fig. 4B). This implies that a small increase in relative fitness per seed can give rise to selection for a considerable decrease in the seed-per-flower ratio. By our definition, the relative fitness per seed for the control series with a seed-to-flower ratio of 0.9 equals one. In addition, the relative fitness per seed for the ovule removal treatment (seed-to-ovule ratio = 0.49) corresponds to a seed-to-flower ratio of 1.97. It appears that this datapoint for the ovule removal treatment is exactly on the theoretical total female fitness isocline. This strongly suggests that the differences in offspring survival that we measured, although small, may be highly relevant for the selection of low seed-to-flower ratios. Of course, we neglected the possible effects of lowering the seed-to-flower ratio on male fitness. It is most likely that including male fitness would push seed-to-flower ratios even lower, because a low seed-to-flower ratio is accompanied by the production of more flowers, which will enhance male fitness per plant.

The quality difference of 14.4% that we found between the control plants and plants of the ovule removal treatment may even be an underestimation of the expected differences in total lifetime fitness. Our estimation of fitness is based only on the survival percentages of the control group and the ovule removal treatment, without taking into account that the seeds of the ovule removal treatment had a higher mass that may have compensated for some of the selective effects. If the differences in lifetime fitness are slightly larger than we detected, selection could act to lower the number of seeds per flower from 2 to 0.9.

For other species for which selective seed abortion has been studied, the trade-off between flower and seed production is not known. Therefore, these species cannot be compared directly to this model. However, to have some idea about the generality of our findings in relation to the model, we can look

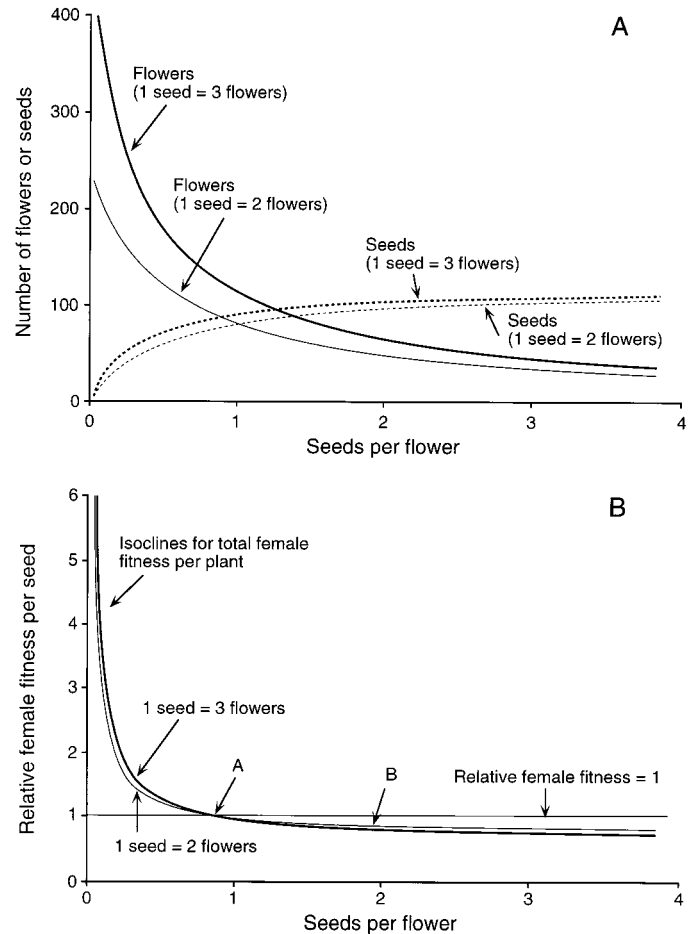


Fig. 4. (A) Flower and seed production at different levels of seed-to-flower ratios, for two different trade-offs between flower and seed production and assuming a fixed amount of resources available for reproduction. (B) Theoretical relative fitnesses per seed that produce equal female fitnesses for plants with different seed-to-flower ratios. Isoclines for total female fitness per plant are plotted for two different trade-offs between seed and flower production and assuming a fixed amount of resources available for reproduction. The relative female fitness per seed for a seed-to-flower ratio that is equal to that of the control group is set to one. Two experimental data curves are plotted: A = mean seed per flower and corresponding relative fitness measured per seed in the control group; B = mean seed per flower (extrapolated from the mean seed per ovule) and corresponding mean relative fitness as measured in the ovule removal treatment for a hypothetical plant with all ovules intact and with a seed-to-ovule ratio similar to that of the ovule removal treatment.

to the results of Casper (1988) about seed abortion in *Cryptantha flava*. *Cryptantha flava* is also a member of the Boraginaceae and bears four ovules in each flower. Although the trade-off between flower and seed production in this species is unknown, it might be similar to the trade-off of *C. officinale*. The control flowers in the study on *C. flava* produced on average 0.88 seeds per flower, which is very similar to the 0.9 seeds per flower observed for *C. officinale*. The line that sets the relative female fitness at one for *C. flava* is thus similar to the one for *C. officinale* in Fig. 4B. In the experimental flowers of *C. flava*, three of the four ovules were randomly destroyed, and seed set was 0.419 seeds per remaining ovule (Casper, 1988). Extrapolated to four ovules per flower, the experimental flowers produced 1.676 seeds per flower. This indicates that in this study on average at least 47.7% [(1.676 - 0.877)/

1.676] of the viable embryos were aborted. The survival of the offspring over a 2-yr period was recorded for five study sites where the offspring were planted. A weighted average over the five study sites shows that survival of the offspring from experimental flowers (0.250, data from Table 1 in Casper 1988) was lower than survival of the offspring from the control flowers (0.297). Size of the seedlings after 1 or 2 yr was not affected by treatment. The relative female fitness of the experimental flowers was then estimated at 84.2% of the female fitness of the control flowers [$1 - (0.297 - 0.250)/0.297$]. This is almost on the female fitness isocline that is depicted in Fig. 4B. Both the difference in seed set per ovule after destruction of ovules and the resulting female fitness are similar to the data found for *C. officinale*.

Previous modelling by Burd (1998) on selective seed abortion generated a saturating female fitness gain curve with increasing flower number. He concluded, therefore, that selective abortion might select for only a small amount of surplus flowers and that higher amounts of surplus flowers were likely attributable to other explanations, e.g., the pollen donation hypothesis. However, taking into account a trade-off between seed and flower production and limited resources, we argue that even if selective abortion causes only relatively small increases in seed quality, this may account for considerable shifts in the mean seed number per flower.

Summary of conclusions—Removing ovules doubled the seed production in the remaining ovule, so abortion of otherwise viable embryos was shown for *C. officinale*. Although seed production was increased by the removal of ovules, it remained still far from 100%. We concluded, therefore, that bet hedging is an unlikely explanation for the low seed-to-flower ratio. The seeds produced in the ovule removal treatment (which had a lower abortion rate than the control series) appeared to be of lower quality in terms of survival, indicating that aborted embryos in the control series have a lower potential fitness than the embryos that are not aborted. We did not find additional effects of maternal selection on the size of the remaining offspring. Small increases in offspring quality, through selective abortion, may have strong selective effects on seed-to-flower ratios.

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