Heritability of nectar production in *Echium vulgare*

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Heritabilities of nectar production in the wild species *Echium vulgare* were estimated as realised heritability under controlled and field conditions. The nectar production of offspring from high- and low-nectar-producing parents was significantly different in both controlled and field conditions, indicating that nectar production is in part genetically determined. The present study is the first one to report a genetic component of variation of nectar production in a wild plant species in the field. Heritability estimated under controlled conditions was 0.13 and therewith less than the heritability estimated under field conditions, which amounted to 0.26. Offspring of high-nectar-producing plants produced comparable amounts of nectar in the growth chamber (1.28 μ l) and in the field

(1.22 μ l). In contrast, the nectar production of offspring of low-nectar-producing plants was significantly higher in the growth chamber (0.95 μ l) than in the field (0.55 μ l), indicating a genotype by environment interaction. The level of heritability of nectar production was dependent on the environment. Under less favourable conditions, like those in the field, heritability of nectar production increased. Nectar production was not correlated with any of the vegetative or reproductive traits measured, and hence no costs of nectar production could be detected. Results obtained stress the importance of field measurements in determining heritabilities.

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Introduction

In animal-pollinated plants, the amount of floral nectar produced is an important trait in the interaction between plants and their pollinators. Nectar volume influences pollinator behaviour as high nectar production may increase the number of pollinator visits (Pleasants, 1981; Galen and Plowright, 1984; Thomson, McKenna and Cruzan, 1989; Real and Rathcke, 1991; Klinkhamer, de Jong and Linnebank, 2001), the number of flowers visited in sequence on the same inflorescence (Heinrich, 1979; Waddington, 1981; Zimmerman, 1983; Cresswell, 1990; Mitchell, 1993; Gonzalez *et al.*, 1995) and the duration of visits in individual flowers (Galen and Plowright, 1984; Cresswell, 1999). Subsequently, changes in pollinator behaviour may increase pollen import and export and thus female and male fitness (Real and Rathcke, 1991; Galen, 1992; Mitchell and Waser, 1992; Mitchell, 1993; Hodges, 1995).

The great variation in nectar rewards among and within plants (Rathcke, 1992, and references therein) and the link between nectar production and plant fitness should subject nectar production to natural selection (Zimmerman, 1988). However, a prerequisite for natural selection to occur is that at least part of the variation is heritable. Most of the knowledge about heritability of nectar reward is based on agricultural studies (Pedersen, 1953; Hawkins, 1971; Teuber and Barnes, 1979; Teuber *et al.*, 1990). Relatively little is known about the heritability of nectar production in wild plants. Substantial heritabilities of

nectar production under controlled conditions, ranging from 0.38 to 0.64, were determined in *Penstemon centranthifolius* (Mitchell and Shaw, 1993), *Epilobium canum* (Boose, 1997) and *Echium vulgare* (Klinkhamer and van der Veen-van Wijk, 1999). Under field conditions Campbell (1996) did not detect any genetic variation of nectar production in *Ipomopsis aggregata*. While the studies under controlled conditions can indicate the presence of genetic variation, they do not necessarily provide reliable estimates of heritabilities under field conditions. Heritabilities are sensitive to effects of the environment on the expression of phenotypic traits (Falconer, 1989). In addition, artificially high heritabilities may be measured under controlled conditions due to a more constant environment with reduced environmental variance compared to wild plants in the field (Campbell, 1996). We therefore estimated heritability of nectar production in the wild plant species *E. vulgare* under controlled and field conditions in this study. Experiments with *E. vulgare* and its most important pollinators, *Bombus terrestris* and *B. pascuorum*, have demonstrated that high nectar production can lead to an increase in the number of pollinator visits (Klinkhamer, de Jong and Linnebank, 2001). Nectar production in *E. vulgare* is sensitive to environmental variation (Corbet, 1978) and shows considerable genetic variation among clones under controlled conditions expressed as a clonal repeatability of 0.48 (Klinkhamer and van der Veen-van Wijk, 1999). However, this estimate is an upper limit of heritability of nectar production in *E. vulgare*. Clonal repeatability includes genetic and non-genetic maternal as well as nonadditive genetic factors (Falconer, 1989).

Little is known about the costs of nectar production, although it is generally assumed that increased nectar production is costly for the plant. Thus the effect of nectar production on fitness depends on the balance

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between its costs and its positive effects on male and female reproduction. Southwick (1984) estimated secretion of nectar sugar in *Asclepias syriaca* at 37% of the daily photosynthate assimilated during the flowering period and nectar production of *Medicago sativa* to be twice the energy invested in seeds. Pleasants and Chaplin (1983) reported that nectar production in *A. quadrifolia* increased with root weight and decreased with large umbel size. There is only one study demonstrating costs of nectar production (Pyke, 1991). Removal of nectar from flowers of *Blandfordia nobilis* increased the plant's net nectar production but reduced its ability to produce seeds. Nectar production in *E. vulgare* did not infer trade-offs against floral traits (Klinkhamer and van der Veen-van Wijk, 1999). We therefore try to locate costs of nectar production causing trade-offs against other vegetative and/or reproductive traits in the current study examining correlations. The questions addressed in the present study are then:

1. What is the realised heritability of nectar production in *E. vulgare* determined under controlled and field conditions?
2. Do heritabilities determined under controlled and field conditions differ?
3. Does nectar production infer costs causing trade-offs against vegetative and/or reproductive traits in *E. vulgare*?

Material and methods

The plant species

The plant species *E. vulgare* (vipers' bugloss) is a self-compatible monocarpic perennial commonly found in open sandy dry habitats. Each plant produces one to 20 inflorescences each with about 50 cymes, which carry up to 20 flowers. In total, one *E. vulgare* plant may produce hundreds of flowers, of which usually on each cyme one to two are open at the same time. The species is gynodioecious. In this study, only hermaphrodite plants were used. In hermaphrodite plants flowers are protandrous. Flowers are about 20 mm long and their petals are fused to form a tube at the bottom of which three nectaries are located. Nectar production commences with the opening of the flowers and continues during the lifetime of the flower, which usually lasts 2 days (Corbet, 1978). The amount of nectar produced 2 h after opening is strongly correlated with the lifetime nectar production of the flower (Klinkhamer and van der Veen-van Wijk, 1999).

Heritability

Rosettes of *E. vulgare* were haphazardly chosen from populations at Meijendel, a sand dune area near The Hague, The Netherlands, during spring 2001. Rosettes were potted in 4.5-l pots filled with an equal mixture of dune sand and potting soil fertilised once with 4 g 15-10-12 NPK slow release fertiliser. The potted plants were grown in a common garden. In all, 60 plants were transferred to a growth chamber (L:D 18:6, 20°C:10°C) when all cymes of at least one inflorescence had open flowers. Plants were randomly assigned to a place in the growth chamber and watered three times a week. The nectar content of a newly opened flower was measured

for each individual plant inserting 1 µl microcapillary tubes. Newly opened flowers can be distinguished from the older ones by their pink colour and their style being shorter than the stamen. After nectar measurement nectar production was ranked from the highest to the lowest volume. This was repeated until the ranking of nectar production was stable, which occurred after six flowers had been measured for each plant. The first five plants of the highest ranking and the last five plants of the lowest ranking were selected as parents. Individuals within each selected group were crossed among each other. To ensure pollination, each group was placed into a separate insect cage (1.60 m × 85 cm × 80 cm) in the growth chamber to which 12 *B. terrestris* bumblebees were added twice. Open flowers during the pollination period were marked.

In total, 12 haphazardly chosen seeds from the marked flowers of each parent plant were germinated in September 2001. Seedlings were potted and pots were placed in a randomised design within a growth chamber. Potting, fertilising and watering followed the regime described above for the parents. Plants were grown in a growth chamber (L:D 16:8, 20°C:20°C) for 8 weeks. Thereafter, six randomly chosen offspring per parent plant were dug in pots into a field plot in Meijendel, while the remaining six offspring per parent plant received a cold treatment in a cold chamber (L:D 8:16, 5°C:5°C) for 11 weeks. After the cold treatment, these plants were transferred back and randomly assigned to a place in the growth chamber (L:D 18:6, 20°C:10°C). All plants in the growth chamber flowered, except five, and three in the field. The nectar production of six randomly chosen newly opened flowers was measured when plants were in full flower and the average value per plant was used in the subsequent analysis. In the field, plants were individually protected with gauze the evening before nectar measurement to prevent pollinator visitation of flowers.

Heritability was determined as realised heritability (Falconer, 1989). Data of nectar production in the field did not fit a normal distribution. Neither did untransformed nor log-transformed data of parental nectar production. While analysis based on changes of mean trait values of log-transformed data gave similar results as analysis based on changes in median trait values, the median was thought to be a more reliable basis for the calculations of realised heritability being a nonparametric statistic. Realised heritability is expressed as the response to selection (R) being a proportion of the selection differential (S) (Falconer, 1989):

$$h^2 = R/S$$

where by

$$R = M_{R+} - M_{R-} \text{ and } S = M_{S+} - M_{S-}$$

where R is the response to selection expressed as the difference of the median phenotypic value of the high- (M_{R+}) and low- (M_{R-}) nectar-producing offspring (Figure 1), and S the selection differential expressed as the difference of the median phenotypic value of the high- (M_{S+}) and low- (M_{S-}) nectar-producing individuals selected as parents. We were not primarily interested in differences between high and low nectar production lines, but to show that there is heritable genetic variation present upon which selection can act. Therefore, high

Figure 1 Frequency distributions of nectar production in *E. vulgare* for the parental generation and for offspring of high- and low-producing parents grown in a growth chamber and in the field after one generation of selection. The determination of the selection differential and the response to selection is demonstrated. The selection differential (S) is expressed as the difference of the median phenotypic value of the high- (M_{S+}) and low- (M_{S-}) producing individuals selected as parents. The response to selection (R) is expressed as the difference of the median phenotypic value of the high- (M_{R+}) and low- (M_{R-}) nectar-producing offspring.

and low nectar production lines were combined for the calculation of heritability to make the calculations independent of the parental median before selection and thus independent of an experimental control (Drent *et al*, 2003; Van Oers *et al*, 2004), which requires crosses among parents with average levels of nectar production. However, parents with average levels of nectar production are most likely to be heterozygous, leading to Mendelian segregation of alleles responsible for nectar production in the following generation.

To compare medians, a nonparametric test was used to test whether two or more independent samples are drawn from populations with the same median using the χ^2 statistic (Dixon and Massey, 1957).

Costs

Four offspring per parent plant were chosen at random from the plants raised in the growth chamber and used for measurement of correlations between nectar production and various vegetative and reproductive traits in *E. vulgare*. Plants were pollinated by *B. terrestris* bumblebees. Flowering of plants was not synchronous and therefore subsequent groups of flowering plants were placed into two insect cages (1.60 m × 85 cm × 80 cm) in the growth chamber to which 12 bumblebees were added. Plants were transferred back and randomly assigned to a place in the growth chamber to allow for seed set after 8 days of pollination. At 8 weeks after first flowering, plants were harvested. Most traits measured, except time to first flowering, the viability of pollen and germination rate, were determined at harvest. Vegetative traits comprised time to first flowering, defined as time between unfolding of the cotyledons and the opening of the first flower, above-ground biomass as dry weight of stems, leaves and flowers, and root biomass as dry weight of roots. The reproductive trait viability of pollen was determined as the mean percentage of five haphazardly chosen freshly opened flowers. Pollen was collected at random from one anther for each of the five flowers per plant, stained with 1% methylene blue and examined under a light microscope at a 10 × 10 magnification. Nonviable pollen grains in *E. vulgare* are desiccated and shrunken in contrast to viable pollen grains. Further reproductive traits measured were the total number of flowers produced per plant, number of mature seeds per flower, seed weight as the average weight of all mature seeds per plant and percentage germination of seeds. To determine germination rates, 50 seeds per plant were placed in 18 cm × 13 cm plastic trays filled with an equal mixture of dune sand and potting soil. These trays were randomly arranged in a growth chamber (L:D 16:8, 20°C:20°C). Germination was recorded after 18 days since most *E. vulgare* seeds germinate within 2 weeks. Data of all traits did fit a normal distribution except for viability of pollen and germination rate, which were arcsine transformed. To test for associations between nectar production and vegetative and/or reproductive traits in *E. vulgare*, phenotypic Pearson correlations, based on individual plants, and genetic Pearson correlations, based on the mean of the offspring of one parent plant, were calculated. *P*-values were corrected with the improved Bonferroni method for multiple comparisons (Haccou and Meelis, 1994).

Results

The nectar production of high- and low-producing parents was significantly different (χ^2 with Yate's continuity correction = 6.40, *df* = 1, *P* = 0.011, *n* = 10). Also, the nectar production of the offspring of high- and low-producing parents differed significantly in both controlled conditions (χ^2 = 6.555, *df* = 1, *P* = 0.010, *n* = 55) and in the field (χ^2 = 14.675, *df* = 1, *P* ≤ 0.001, *n* = 57), indicating that nectar production is in part genetically determined. The estimated heritabilities are given in Table 1. The heritability measured in the growth chamber was less than the heritability measured in the field.

The frequency distributions of the nectar production of parents and offspring grown under controlled and field conditions are presented in Figure 1. Environment did not affect offspring nectar production comparing the overall median of nectar production measured in the growth chamber and in the field, respectively (χ^2 = 1.751, *df* = 1, *P* = 0.186, *n* = 112) (Table 2). Analysing offspring of high and low nectar production separately, the median of high-nectar-producing plants did not differ between growth chamber and field (χ^2 = 0.074, *df* = 1, *P* = 0.785, *n* = 54), while the median of low-nectar-producing plants in the growth chamber was significantly larger (χ^2 = 6.905, *df* = 1, *P* = 0.009, *n* = 58) than that of plants in the field.

The viability of pollen was genetically positively correlated with above-ground biomass (*r* = 0.897, *P* ≤ 0.001, α = 0.00139, *n* = 10). Phenotypically this correlation was only significant before Bonferroni correction for multiple comparisons. No significant correlations of nectar production and any of the vegetative and reproductive traits measured could be detected either with respect to phenotypic or genetic correlations (Table 3).

Discussion

To our knowledge, the present study is the first one to report a genetic component of variation of nectar production in a wild plant species in the field. Heritability measured under controlled conditions, being

Table 1 Response to selection (*R*), selection differential (*S*) and heritability (*h*²) of nectar production (μl) in *E. vulgare* measured in a growth chamber and in the field after one generation of selection

	<i>R</i>	<i>S</i>	<i>h</i> ²
Growth chamber	0.33	2.56	0.13
Field	0.67	2.56	0.26

Note that the selection differential is based on the parental generation and was therefore measured only once.

Table 2 Median (μl) of nectar-production rates in *E. vulgare* measured in a growth chamber and in the field after one generation of selection

	High and low nectar production	High nectar production	Low nectar production
Growth chamber	1.05	1.28	0.95
Field	0.86	1.22	0.55

Table 3 Phenotypic (based on individual plants $n = 40$) and genetic (based on the mean of four offspring of one parent plant $n = 10$) Pearson correlations between nectar production rate and various vegetative and reproductive traits in *E. vulgare*

Trait	Phenotypic correlation with nectar production	Genetic correlation with nectar production
Time to first flowering	−0.41 P = 0.805 $\alpha = 0.0100$	0.103 P = 0.776 $\alpha = 0.01667$
Above-ground biomass	−0.171 P = 0.299 $\alpha = 0.00278$	−0.444 P = 0.198 $\alpha = 0.00192$
Root biomass	−0.252 P = 0.122 $\alpha = 0.00178$	−0.165 P = 0.648 $\alpha = 0.00714$
Viability of pollen	0.104 P = 0.530 $\alpha = 0.00333$	−0.621 P = 0.056 $\alpha = 0.00156$
No. of flowers	0.202 P = 0.217 $\alpha = 0.00238$	0.365 P = 0.300 $\alpha = 0.00250$
No. of seeds per flower	−0.160 P = 0.331 $\alpha = 0.00294$	−0.150 P = 0.678 $\alpha = 0.00833$
Seed weight	−0.084 P = 0.610 $\alpha = 0.00455$	−0.301 P = 0.398 $\alpha = 0.00333$
Germination rate	0.237 P = 0.171 $\alpha = 0.00208$	0.047 P = 0.897 $\alpha = 0.02500$

Levels of significance before (P) and after correction (α) according to the improved Bonferroni method for multiple comparisons are presented.

0.13, was relatively low. Klinkhamer and van der Veen-van Wijk (1999) reported a heritability of nectar production in *E. vulgare* of 0.48, Boose (1997) of 0.64 in *Epilobium canum* and Mitchell and Shaw (1993) of 0.53 in *P. centranthifolius* under controlled conditions. However, all these were broad-sense heritabilities determined as clonal repeatabilities, providing an estimate of the upper limit of heritability of nectar production, including possible genetic and nongenetic maternal factors as well as other nonadditive genetic factors (Falconer 1989). Comparing our estimate to the narrow-sense heritability of 0.38 in *P. centranthifolius* under controlled conditions reported by Mitchell and Shaw (1993), it is still low. This might be due to the estimation of realised heritability based on one generation of selection in view of the relative long generation time of *E. vulgare*. Use of one generation of selection leads, in general, to a somewhat reduced heritability caused by the nonrandom mating of selected parents, resulting in a negative population disequilibrium reducing additive genetic variance (Falconer, 1989).

Although it is generally stated that measurements under controlled conditions with reduced environmental variance lead to higher heritability estimates, the heritability determined in the growth chamber in the present

study was lower than that determined in the field. For offspring of high-nectar-producing plants, both environments were comparable leading to the same nectar production. A further increase of nectar production is probably only limited by physiological constraints. In contrast, the nectar production of offspring from low-producing plants was higher under controlled conditions than in the field, indicating a genotype by environment interaction. Such genotype by environment interactions have also been demonstrated for nectar production in *Epilobium canum* grown under different light and water regimes (Boose, 1997). Teuber and Barnes (1979) reported for *M. sativa* that crosses of high-nectar-producing plants did result in high-producing offspring, while crosses of low-producing plants did not necessarily result in low production plants. Although nectar production in *E. vulgare* had a heritable component, the level of heritability was dependent on the environment. Under less favourable environmental conditions, like those in the field, heritability of nectar production increased.

Given its phenotypic variation and heritability selection of nectar production in *E. vulgare* seems possible, although the genotype by environment interaction may slow down selection. Whether higher nectar production, which increases pollinator visits in *E. vulgare* (Klinkhamer et al, 2001), leads to increased female and/or male fitness is currently under investigation.

The viability of pollen was positively correlated to plant above-ground biomass. Given the relatively constant environment in the growth-chamber, genotypes producing large biomass might be the relatively better genotypes also expressing higher viability of pollen. Neither phenotypic nor genetic correlations between nectar production and any of the vegetative or reproductive traits measured could be established. Klinkhamer and van der Veen-van Wijk (1999) detected a positive correlation between nectar production and nectar sugar concentration in *E. vulgare*, but no significant correlation between nectar production and measures of flower size and pollen production. Thus so far there is no evidence for costs of nectar production in *E. vulgare* causing trade-offs. Perhaps trade-offs are linked to other vegetative and/or reproductive traits not measured in the present study. Conversely, our design might have lacked power to detect significant correlations. Clearly, costs causing potential trade-offs of nectar production in *E. vulgare* need further investigation.

The present study is the first one to report a genetic component of variation of nectar production in a wild plant species in the field. The level of heritability of the nectar production of *E. vulgare* was dependent on the environment. Under less favourable conditions, like those in the field, heritability of nectar production increased. Results obtained stress the importance of field measurements in determining heritabilities.

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