Searching and Breeding for Structural Features of Flowers Correlated with High Nectar-Carbohydrate Production

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Abstract

Structural features of flowers correlated with high nectar-carbohydrate production are examined, particularly from studies of various agricultural and horticultural plants. Some of these floral traits, and nectar characteristics (volume, and sugar concentration and production) themselves are followed in studies of inheritance. Recommendations are given for characteristics indicative of high nectar yields, and for those traits best eliminated early in breeding programmes.

INTRODUCTION

Nectar is an attractive source of food for many species of flower-visiting animals, particularly insects. Of prime importance to agronomists and horticulturalists, these nectar-gathering visitors may become dusted with pollen and hence often serve as pollinators essential for fruit and seed set. Moreover, honeybees (*Apis* spp.) convert nectar into a commercially important, worldwide sweetener, honey. Not surprising, therefore, is the growing interest in the understanding of floral structure, nectaries and the mechanisms of nectar secretion in the context of selection for high nectar production.

It is well established for a large number of species that individual plants, even within a cultivar or among selections, can regularly vary by 2- to 4-fold in terms of quantity of nectar sugar produced (Shuel 1989). Indeed, in the legumes alfalfa (lucerne) (*Medicago sativa*), soybean (*Glycine max*) and birdsfoot trefoil (*Lotus corniculatus* and related species), differences of 12.3-fold (Barnes and Furgala 1978), 8.9-fold (Severson and Erickson 1984) and 7.0-fold (Campbell and Ayers 1989) were recorded in nectar carbohydrate per flower between selections, cultivars and accessions, respectively. Accordingly, the existence of considerable intraspecific variability in nectar production allows improvements by classical breeding to be realized (Shuel 1989).

Nectar sugar is also a relatively inexpensive reward to offer potential pollinators. A rich sucrose solution contains only 40% or less of the energy contained in the same quantity of pollen or oil (Simpson and Neff 1983). And while 30% or more of a milkweed (*Asclepias* spp.) plant's daily photosynthate is required for nectar-sugar production (Pleasants and Chaplin 1983, Southwick 1984), nectar comprised only 3.3% of the energy content of a pickerelweed (*Pontederia cordata*) flower and seems typical for many other bee-visited species, suggesting nectar production is "a minor component of the energy expended on flowering" (Harder and Barrett 1992). These facts support the research interest in enhancing the attractiveness of crop plants to insect pollinators, and at the same time improving honey-production potential, through plant breeding for increased nectar-carbohydrate production.

Recent studies in non-agricultural settings have evaluated some of the costs and consequences to pollination of increased nectar production by plants. In *Blandfordia nobilis* (Liliaceae), fewer seeds were set in plants from which more nectar sugar had been removed (Pyke 1991). Flowers augmented with up to twice the natural level of nectar in *Mirabilis multiflora* (Nyctaginaceae) increased hawkmoth visitation, pollen deposition and removal, and intraplant movements which led to elevated self-pollination, yet reduced seed set (Hodges 1995). Other studies involving bumblebees, hawkmoths and hummingbirds have recorded greater numbers of visits to flowers in response to increased nectar volumes (see Hodges 1995). For the orchid, *Comparettia falcata*, however, artificially-increased nectar volumes did not alter hummingbird visitation, self-pollination

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or reproductive success (Salguero-Faria and Ackerman 1999). More research of this kind is needed for agricultural crops, too. But if alfalfa is typical, even if high nectar yields per floret were available many visits would still be required (probably to several plants in high-density plantings) before an individual honeybee became nectar-laden. Vansell (1941) found that nectar quantity ranged from 0.83 - 2.4 µL per floret, allowing foraging bees to fill their honey sacs after 15 - 45 visits. In reality, even these high numbers of visits needed before a honeybee is satiated are too low, because they assume maximal standing quantities of nectar available in each floret, which is unlikely.

The reader is referred to the excellent article by Shuel (1989) for explanations of key elements about breeding for nectar production. Owing to the laborious nature often involved in nectar collection itself (Kamler 1984), this treatise places emphasis on assessment of structural features of the flower and nectary (section 2) that are indicative of high nectar-carbohydrate production in agricultural and horticultural crops. Finally, studies of nectar inheritance (section 3) are reviewed and recommendations made (section 4) regarding floral traits best correlated with nectar production.

STRUCTURAL FEATURES OF FLOWERS EXAMINED IN RELATION TO NECTAR YIELD

Due to our incomplete understanding of the process of nectar secretion, most structural characters investigated in reference to floral nectar production have an empirical basis. Below, these structural traits have been designated as pertaining to flower size (section 2.1), the flower stalk (2.2), the flower base (2.3) and features of the floral nectaries (2.4). Overall, more research has been conducted with species of legumes than any other taxon.

Flower Size

Among the morphological features of flowers that have been examined in relation to nectar yield include visual assessments of flower size, flower weights, plus measurements of the corolla and length of the gynoecium.

1. Visual Score of Flower Size. Various germplasm sources of alfalfa (*Medicago sativa*), when rated visually for size of their florets, gave a significant correlation ($r = 0.52$) with nectar volume (Barnes and Furgala 1978). In breeding programmes for increased nectar volume, preliminary screening by eyesight is advocated (Barnes and Furgala 1978, Teuber and Barnes 1979).

2. Floral Biomass. For a large number of plant species representing various pollination syndromes, Opler (1983) determined that maximum available nectar per flower is highly correlated ($r = 0.99$, $P < 0.001$) with fresh weight of the flower.

3. Size of the Corolla. In red clover (*Trifolium pratense*), a positive correlation ($r = 0.455$, $P < 0.001$) existed between height of the nectar column and length of the floret's corolla tube (Bond 1968).

Reductions in planting density of *Medicago sativa* led to larger florets. Plants grown in hills had florets with standard petals 8.08 mm long and produced 0.21 µL nectar, whereas plants in dense stands had smaller petals (6.68 mm) and reduced nectar volumes (0.10 µL) (Pedersen 1956). Vansell (1941) also reported a relationship between floret size and nectar volume in this species.

In eight varieties of another legume, *Lotus corniculatus*, area of the standard petal was correlated positively with nectar volume per floret ($r = 0.358$, $P < 0.05$). Compared to other structural features (see section 2.2), this relationship between petal area and nectar volume was considered too weak to recommend for selection (Murrell et al. 1982a).

Significant differences in both petal length and width (Davis et al. 1996) were correlated positively with average nectar-sugar yield per flower (Davis et al. 1994, 1995) in haploid (n), diploid (2n) and tetraploid (4n) lines of *Brassica rapa*.

Among 13 species of *Scrophularia*, corolla size was correlated strongly ($r = 0.92$, $P < 0.001$) with nectar volume (Ortega Olivencia and Devesa 1993).

On the contrary, no correlation between either length or width of the corolla and

nectar volume or sugar production occurred in *Penstemon centranthifolius* (Scrophulariaceae) (Mitchell and Shaw 1993). Hodges (1993) found significant phenotypic correlations to be absent between perianth length and nectar volume (r = 0.25), nectar-sugar concentration (r = 0.06) and total-nectar sugar (r = 0.26) in flowers of *Mirabilis multiflora*. Furthermore, in different species and cultivars of watermelon (*Citrullus* spp.), Wolf *et al.* (1999) found no relationship between corolla diameter and number of honey bee visits (r = -0.05), nor between the former and nectar volume or sugar concentration.

4. Length of the Gynoecium. In five selections of a variety of broad bean (*Vicia faba*), the lengths of the immature beans measured from the receptacle base to stigma tip of mature, nectar-yielding flowers were compared with data for total nectar sugar (Davis and Gunning 1991, Davis 1992). Bean length and nectar-sugar production were greatest in selection K, but selections A and E with the next-longest gynoecia produced relatively low quantities of nectar sugar (Table 1).

Similar results were obtained in non-agricultural species. Mitchell and Shaw (1993) found no correlation between gynoecium length of *Penstemon centranthifolius* and volume (r = 0.07) or sugar content (r = 0.11) of floral nectar. No significant correlation existed between style length and nectar volume (r = 0.09), nectar-sugar concentration (r = -0.05) or total nectar sugar (r = 0.06) in flowers of *Mirabilis multiflora* (Hodges 1993).

Flower Stalk (peduncle)

As nectar is derived from the plant's phloem sap (Frey-Wyssling 1955), it seems worthy to compare floral nectar production with characteristics of the vascular supply entering the flower base through the peduncle. Evidently, structural features of the flower stalk have been related to nectar yields in only a single species, *Lotus corniculatus*. Murrell et al. (1982a) compared nectar volumes per inflorescence to peduncle size and to characteristics of the phloem supply located within the peduncle.

1. Cross-Sectional Area. From six varietal selections chosen on the basis of nectar production (three high-yielding, and three low-yielding), nectar yield per umbel was correlated strongly with the cross-sectional area of the peduncle (Fig. 1).

2. Vascular Supply. Nectar yield per inflorescence also was correlated positively and significantly with the total number of vascular bundles located within the flower stalk (Fig. 2). Quantification of functional (transport) phloem within the vascular bundles also was revealing. When nectar yield per umbel was compared with the cross-sectional area of phloem within each peduncle, a strongly positive and significant correlation resulted (Fig. 3). Although reliant on microscopy, this laboratory assessment of peduncle phloem may be a very valuable technique for estimating nectar-yield potential during screening. Fortuitously, the cross-sectional area of functional phloem and cross-sectional area of the peduncle (see above) are themselves closely correlated ($r > 0.9$, $P < 0.01$) (Murrell et al. 1982a).

Flower Base

More attention in selection for nectar production has been given to structural features of the flower base than to the flower stalk. Included are size of the receptacle, and diameter of the "nectar reservoir" of legumes.

1. Receptacle Size. A template with holes drilled to accommodate increasing sizes of receptacles has been utilized in the field to rapidly screen accessions of three species of *Lotus* (Campbell and Ayers 1989). The potential usefulness of these parameters of receptacle size, namely diameter and height (and from them, calculations of receptacle volume), to estimate total nectar carbohydrate per floret, has been determined. For *L. caucasicus* and *L. tenuis*, quantities of nectar sugar were best correlated with receptacle volume (r = 0.526 and 0.630, respectively), whereas the best indicator of nectar carbohydrate per floret in *L. corniculatus* was receptacle height (Campbell and Ayers 1989).

In *Medicago sativa*, correlations between nectar volume and diameter of the

receptacle were also significant and positive for all three cultivars examined on an individual-plant or plot-mean basis (Table 2, top half) (Teuber et al. 1983).

Furthermore, for eight species of Lamiaceae, Dafni et al. (1988) found a strong correlation (r = 0.97) between nectar quantity per flower and volume of the receptacle.

2. Nectar Reservoir. Typical of many papilionaceous legumes is the presence of a hollow in the region of the bases of the free stamen and ovary, the greatest distance of which has been referred to as the diameter of the "nectar reservoir" (see Fig. 1B, C of Teuber et al. 1983). This cavity forms from the staminal column and standard petal and fills with nectar (Teuber et al. 1980). If the nectar data from three clones (high-, medium- and low-nectar yielding) for each of two cultivars of alfalfa are plotted against diameter of the nectar reservoir of their florets (Teuber et al. 1980), a strongly positive and significant correlation results (Fig. 4). Clones producing the most nectar per floret had the largest nectar-reservoir diameters.

Nectary Features

In addition to relationships of various floral structures with nectar production, structural features of the floral nectaries themselves have been investigated. Studies have focussed on the volume and surface area of the nectar glands, features of the modified stomata common on nectary surfaces of many taxa, and the vascular supply to the nectary.

1. Nectary Size. To date, results of studies that have related nectary size to nectar production have not allowed definitive generalizations to be drawn. In a survey of 13 legume species, Gulyás and Kincsek (1982) showed that nectar sugar per flower was correlated directly with the volume of the floral nectary (Fig. 5). On the other hand, in another interspecific comparison the area of nectary surface among four species of Rosaceae was not correlated with nectar sugar quantity per flower (Weryszko-Chmielewska et al. 1997). Flowers of *Aronia melanocarpa*, having the smallest nectary surface, produced relatively large amounts of nectar sugar, whereas flowers with the largest nectary surface (*Cydonia oblonga*) produced a relatively small quantity of nectar sugar (Table 3, top).

Disparate results within these same two families can be found again in studies that focussed instead on nectary size within a species. In an intraspecific study of eight clones of sour cherry (*Cerasus vulgaris* cv. *Pándy*), the area of glandular surface on the hypanthium was correlated with nectar-sugar production (Orosz-Kovács and Gulyás 1989). On the contrary, in five selections of *Vicia faba* cv. *Aquadulce*, the floral nectaries with the longest protuberances (selection A) produced the least nectar sugar per flower, whereas flowers of the best nectar-yielding selection (K) had nectary projections of intermediate length (Davis and Gunning 1991).

By omitting one species of high nectar-production capacity from the analysis, Dafni et al. (1988) found a significant correlation (r = 0.98) between volume of the nectariferous tissue and nectar yield per flower for eight species of mints and suggested that such relationships mainly occur in closely-related plants with similar nectary anatomy. "The larger the nectary, the more nectar it secretes" (Dafni et al. 1988). Gulyás (1967) showed that volume of the floral nectary among six species of *Lamium* varied up to 22-fold, and Andreev (cited by Beutler 1953) found that floral nectaries at the top of *Phacelia* inflorescences were only 59-63% as long as those formed at the inflorescence base.

Similarly, by ignoring one exception (*Valencia* orange), a close, positive correlation exists between nectary volume and dry weight of the nectar secreted by 10 species or varieties of *Citrus* (Fahn 1949).

Moreover, it is suggested that the larger quantities (2.7-5.5) of nectar sugar produced by pistillate over staminate flowers in three species of the Cucurbitaceae may be related to volume of the nectary tissue in each flower type (Fahn 1949). Reduction in nectary size and the occurrence of collapsed cells and less stored starch in the glandular tissue accounted for the reduction or absence of nectar production in the "Nectarless"

mutant of muskmelon (Bohn and Mann 1960). "Nectarless" is caused by a single recessive gene possibly linked to plant resistance to powdery mildew disease (Bohn 1961).

In a study comparing floral nectar production with increasing levels of ploidy, average volume of the lateral nectaries (associated with the short stamens) was correlated positively (r = 0.803) with mean quantity of nectar carbohydrate per flower between n, 2n and 4n lines of *Brassica rapa* (Davis et al. 1994). Lateral glands of haploid plants averaged only 25-38% the size of those of 2n and 4n plants (Davis et al. 1996) and produced only 28-32% as much nectar carbohydrate (Davis et al. 1994). However, although flowers of 4n *B. rapa* had significantly larger lateral nectaries than those of 2n lines (Davis *et al.* 1996), mean nectar-sugar production per flower did not differ between them (Davis *et al.* 1994). It was concluded that absolute size of the nectaries is not the prime factor dictating nectar-production potential in that species (Davis et al. 1994). Kamler (1984) reported that the size (width and length) of floral nectaries of *B. napus* cv. *arvensis* accorded with the amount of nectar secreted, but sizes were not provided.

Nectary size and morphology can vary within a plant, and even within the same *Brassica* flower (Leung et al. 1983, Davis et al. 1996). An interesting but unexplained outcome of the study of nectary size in *B. rapa* was that, within a ploidy level, plants whose flowers produced the most nectar sugar tended to have the best match in size of their lateral nectaries at opposite sides of the flower (Davis et al. 1994). The highest nectar-sugar yields per flower occurred when the pair of lateral glands were almost equal in size, differing only 1.1-1.2 times in volume, on average.

2. Modified Stomata on Nectary Surfaces.

Number and Density. Comparisons also have been made between nectar production per flower and features of the modified stomata found on the nectary surface, typical of the legumes (Ancibor 1969, Waddle and Lersten 1973). From *Medicago sativa*, the data recorded by Teuber et al. (1980) for clones from each of two cultivars shown in Table 4, do not permit clear interpretation. In cv. *Team*, the number of nectary stomata per floret was correlated positively with nectar volume; however, no such relationship existed between the three clones of cv. *Vernal*. About a 4-fold difference in mean number of nectary stomata per floret occurred between clones (Table 4).

From six varietal selections of *Lotus corniculatus*, Murrell et al. (1982a) found the number of nectary stomata per floret to be correlated negatively with nectar volume per umbel (Fig. 6), though the relationship was not significant. The average number of nectary stomata per floret differed 2-fold between selections.

hereas the floral nectaries of these two species possess concave surfaces between the androecium and gynoecium common to many legume species (Waddle and Lersten 1973, Gulyás and Kincsek 1982), other legumes like those of several species of *Vicia* (Stpiczynska 1995) possess floral nectaries as outgrowths or protuberances (Fig. 7a). Davis and Gunning (1991) found that mean numbers of modified stomata per nectary in broad-bean flowers varied up to 13-fold between four varieties and, within five selections of *Vicia faba* cv. *Aquadulce*, about 3-fold. In the latter cultivar, a negative correlation ($r = -0.84$, $0.10 > P > 0.05$) existed between nectar-sugar production per flower and total number of modified stomata per floral nectary.

In all three of these studies of legumes, the plants with the highest number of nectary stomata actually have produced the least nectar (Table 4; Fig. 6) or nectar sugar (Davis and Gunning 1991) per flower. Similar results for stomatal number per nectary or for nectary-stomatal density now have been found for non-legumes. Among 10 species of *Nicotiana* the number of stomata per nectary varied 5.8-fold (Cocucci and Galetto 1992) and between two species of *Mandevilla*, 5.3-fold (Galetto 1997). Of the four species of Rosaceae listed in Table 3 (top), flowers of *Chaenomeles speciosa* produced more nectar sugar than the others possessing higher stomatal densities; the number of stomata per mm^2 of nectary surface varied up to 21-fold (Weryszko-Chmielewska et al. 1997). In a study of three species of *Eucalyptus* (Davis 1997), the stomatal density varied 2.1-fold between *E. cosmophylla* and *E. pulverulenta* that yielded similar peak quantities of nectar

sugar per bagged flower, and a 5.6-fold difference in the latter parameter between *E. cosmophylla* and *E. grandis* occurred despite similar stomatal frequencies on the nectary surface (Table 3, bottom). In n, 2n and 4n lines of *Brassica rapa*, the lateral nectaries with the most stomata produced the lowest quantities of nectar sugar per flower (A.R. Davis, J.C. Paradis and J.D. Pylatuik, unpublished). An extreme example of this inverse relationship within the Brassicaceae can be found in *Sinapis arvensis*, wherein the median pair of nectaries per flower possessed 2.4-fold as many modified stomata as the lateral pair, yet yielded only 8% of the flower's total nectar sugar (Davis *et al*. 1998).

On the contrary, Sammataro et al. (1985) stated that for varieties of sunflower (*Helianthus annuus*) and related species, more nectary stomata occurred in genotypes highly attractive to honey bees. Stomatal data were not provided, however.

Distribution. Interestingly, not only the number and density, but the distribution of modified stomata per nectary can vary considerably. In broad-bean flowers of selection A, whose floral nectaries possessed the longest projections (see 2.4.1), the highest numbers of modified stomata and the lowest quantities of nectar sugar per flower (Davis and Gunning 1991), had 65% of all modified stomata located on the basal ¾ of the gynoecial (Fig. 7b) and staminal (Fig. 7d) faces of nectary projections. The modified stomata on the faces were usually solitary (Fig. 7b, d) but commonly in groups where up to 17 were connected (Fig.7c). Flowers of selection K, which averaged the highest quantities of nectar sugar (4.4-fold more than those of selection A), had 82% of the total modified stomata located on the distal ¼ of the nectary projection (Fig. 7a, e; Davis and Gunning 1991).

On the other hand, in sunflower, the occurrence of modified stomata predominantly on the nectary tip may be indicative of reduced nectar production. For example, lines CMS89 and RHA274 were highly attractive to bees and reportedly had stomata distributed along the rim and both the inner and outer walls of the nectar gland, whereas florets of the unattractive line CMS290 bore their modified stomata especially on the nectary rim (Sammataro et al. 1985).

Concluding Remarks. Reliance on features of the stomata on floral nectaries for the purposes of selection for high nectar-carbohydrate production is tenuous, judging from the discussion above and accumulating developmental, anatomical and physiological evidence from these stomata. Much of the latter knowledge has accrued from studies of agronomically-important legumes (Waddle and Lersten 1973), including *Medicago sativa* (Teuber et al. 1980), *Vicia faba* (Davis and Gunning 1992, 1993) and *Pisum sativum* (Razem 1998, Razem and Davis 1999). These studies, supported by others of non-legumes (Zandonella 1967, O'Brien et al. 1996, Davis 1997), have demonstrated that, unlike foliar stomata, the pores do not open and close by guard-cell movements and hence do not permit a fine regulation of nectar flow through the pores. In some nectaries, not all nectar necessarily escapes through stomatal pores present (Beardsell et al. 1989). Furthermore, not all mature stomata on the nectary surface participate as exits for nectar (Beardsell et al. 1989; Gaffal et al. 1998), suggesting there are more stomata formed than utilized. So long as stomatal number and pore size are sufficient to allow nectar flow unimpeded, then measures of absolute stomatal number and density may not be dependable as indicators of potential nectar-sugar yields. And the passive involvement in nectar secretion of modified stomata based on species studied to date, also makes reliance on stomatal features difficult to recommend. Indeed, with more than 1,050 species representing over 80 families of flowering plants already known to possess nectary stomata (reviewed by Davis 1992), in only a small proportion of taxa have these structures been examined in detail.

In addition, whereas our understanding of the significance of the number, density, and distribution of stomata on the nectary surface is increasing gradually, still very little is known about the molecular, genetic and environmental factors that control these stomatal features on nectaries.

3. Nectary Vascular Supply. Floral nectaries may possess their own vascular supply, and differences in nectary innervation between taxa are well known. Of 366 species from

many different families surveyed by Frei (1955) and Kartashova (1965), Fahn (1979) summarized that 39.6% lacked nectary vascularization, 47.8% were supplied with phloem alone, and only 12.6% received both xylem and phloem. The type of nectary vascularization may vary within the same family (Fahn 1979) or even between species of the same genus e.g., *Centaurea* (Frei 1955), *Salvia* (Dafni et al. 1988).

The possession of more than one nectary type and location per flower, of marked differences in nectar-production capacity, has allowed comparisons on nectary vascular supply in several species of the Brassicaceae. In over 40 species examined to date, floral nectaries of this family are all supplied by phloem only (see Davis et al. 1998). However, the pairs of nectaries receive their direct supply of phloem differentially. For example, in *Brassica* spp., the lateral pair of nectaries produces 90-96% of a flower's total nectar carbohydrate (Búrquez and Corbet 1991, Davis *et al.* 1998) and receives a rich supply of phloem far into the gland interior, whereas the poorly-yielding median pair receives much less phloem and usually barely beyond the nectary base (Frei 1955, Davis *et al.* 1994, 1998). Results such as these suggest that quantification of nectary vascular supply may be useful for selection purposes.

STUDIES OF INHERITANCE OF NECTAR PRODUCTION

Breeding studies involving nectar volume, concentration or nectar-sugar production have been conducted in at least 10 taxa, nine being of agricultural or horticultural significance. Whereas most studies have focussed on these parameters of nectar production themselves, a few have followed the inheritance pattern of nectar production by breeding for structural features of flowers correlated with nectar yield.

Breeding Based on Structural Features of Flowers

Utilization of morphological features of flowers to study the heritability of nectar characteristics apparently has been limited to two species, alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*), both legumes.

In alfalfa, Teuber et al. (1983) showed that nectar volume and diameter of the floret receptacle were correlated significantly (Table 2, top half), but the correlation remained significant only for subpopulations of cultivars *CUF-101* and *Vernal* selected for nectar volume, and never for subpopulations selected for receptacle diameter, when correlations were calculated for plot means within their selected subpopulations (Table 2, bottom half). The response to selection for receptacle diameter was under additive genetic control, but selection on this trait had no consistent effect on nectar volume; selection for receptacle diameter only moved that characteristic in the direction of selection (Teuber et al. 1983). It was concluded that selection for receptacle diameter of alfalfa florets would, unfortunately, only be marginally effective in improving nectar volume (Teuber et al. 1983). These results from greenhouse studies were supported by those obtained in the field; selection for low nectar volume decreased receptacle diameter but selection for high nectar volume did not affect receptacle size (Teuber et al. 1990).

Breeding projects involving red clover were stimulated by efforts to increase pollination by honeybees. The likelihood of success was deemed high if nectar volume accumulating in the floret's corolla tube could reach within 7 mm of the tube's mouth, that distance approximating the length of the honeybee proboscis (Hawkins 1971). Corolla-tube length and nectar height in the tube were correlated (Bond 1968) and shown to be highly heritable characters governed by additive effects, but improvements in both nectar height and its accessibility (by reduction in corolla-tube length) could occur (Bond and Fyfe 1968). Hawkins (1971) concentrated on nectar height alone. Starting with a population having average corolla-length of 9.8 mm and height of nectar within the tube of 1.45 mm, after four years (generations) of continual selection the mean nectar height had risen by 0.76 mm such that 45% of florets produced sufficient nectar within 7 mm of the corolla-tube's mouth, compared to only 6% of florets in the original population. These increases in nectar column had occurred without change to other plant characteristics (Hawkins 1971).

Breeding Based on Nectar Volume or Nectar-Sugar Production

Features of the inheritance of parameters of floral nectar production themselves have been investigated in a modest number of species. Most progress has occurred in studies investigating nectar volume or nectar-sugar production; the limited genetic variation in nectar-sugar concentration (Shuel 1952) may explain the non-significant differences and low genetic correlations and heritability of this character (Vear et al. 1990, Mitchell and Shaw 1993, Rabinowitch et al. 1993).

1. Breeding Within a Species. Self-pollination of high nectar-producing lines in alfalfa (Pedersen 1953) and *Brassica napus* cv. *arvensis* (Kamler 1984) resulted in high-producing progeny, whereas selfing of low nectar-producing lines gave low-yielding progeny.

Cross-pollination among high nectar-producing lines derived from different varieties gave the highest-yielding progeny in alfalfa (Teuber and Barnes 1979, Teuber et al. 1983, 1990) and birdsfoot trefoil (Murrell et al. 1982b). The opposite occurred in low x low crosses (Murrell et al. 1982b, Teuber et al. 1983, 1990).

Crosses among lines producing high and low nectar volumes resulted in progeny that produced nectar quantities intermediate to those of their parents in alfalfa (Pedersen 1953, Teuber and Barnes 1979), birdsfoot trefoil (Murrell et al. 1982b), pepper (Rabinowitch et al. 1993), sunflower (Vear et al. 1990) and watermelon (*Citrullus lanatus*) (Wolf et al. 1999). Normal frequency distributions in nectar-volume production were obtained in these hybrids (Pedersen 1953, 1956).

Vear et al. (1990) found only slight evidence for heterosis, the average nectar volume produced by the hybrids (0.13 µL) being similar to that of the parents (0.11 µL). Also, results of reciprocal crosses were similar (Pedersen 1953, Teuber and Barnes 1979, Murrell et al. 1982b), indicating both maternal and paternal sources contribute to nectar-production capacity of the progeny.

Pedersen (1953) found a correlation coefficient of 0.833 for the comparison of nectar volume between maternal parent and polycross progeny. Murrell et al. (1982b) found a highly significant correlation (r = 0.96) between the progeny of a diallel cross and the midparent values of nectar volume. Vear *et al.* (1990) also reported a high correlation (r = 0.71) between their five sunflower inbreds and the means of hybrids obtained, though its lack of significance could be attributable to the likeness in nectar volume produced by four of the five parent lines (Vear et al. 1990).

From these outcomes it has been concluded that inheritance of nectar volume behaves in a quantitative (multifactorial) manner, being governed by additive gene effects (Pedersen 1953, 1956, Teuber and Barnes 1979, Murrell et al. 1982b). To date, it has not been possible to estimate the number of genes involved in the control of nectar production (Murrell et al. 1982b). Differences in gene frequency may account for differences in size of variance of general combining-ability effects arising between varieties and clones (Teuber and Barnes 1979).

Estimates of heritability for nectar volume are widespread, ranging from 93% in birdsfoot trefoil (Murrell et al. 1982b), 64% in alfalfa (Pedersen 1953), 38 to 53% in *Penstemon centranthifolius* (Mitchell and Shaw 1993), to 12% in *Capsicum annuum* (Rabinowitch et al. 1993). From their low estimate of heritability, Rabinowitch *et al.* (1993) concluded that selection for high nectar volume in pepper would require the screening of large populations under a variety of environmental conditions.

Fewer estimates for heritability are available for nectar-sugar production and nectar-sugar concentration. In *P. centranthifolius*, the heritability estimate for nectar-sugar production was 37 to 47% and significant, whereas for nectar concentration the estimate was 0% (Mitchell and Shaw 1993)

2. Interspecific Breeding. It seems few studies have investigated total nectar production in hybrids formed between species (Galetto and Bernardello 1995). When the watermelon cultivar BAG (*Citrullus lanatus*) was crossed with L-224 (*C. colycynthis* x *C. lanatus* cv. Mallali), nectar volumes intermediate to those of the parents were obtained (Wolf et al.

1999). From flowers of a natural hybrid (*Lycium ciliatum* x *L. cestroides*), Galetto and Bernardello (1995) recorded nectar volume and sugar quantities that were intermediate to those of the parent species, again common to the outcomes reported above. However, in the hybrid formed by crossing two clover species, *Trifolium nigrescens* with *T. repens*, nectar-sugar production exceeded that of both parents owing to a relatively high sugar concentration (not significantly different from *T. nigrescens*) and a high nectar volume (similar to *T. repens*) in the hybrid (Marshall et al. 1995). Similarly, Beutler (1953) stated that in 1886, Stadler found hybrids from two passion-flower species, *Passiflora coerulea* and *P. alata*, yielded more nectar than the parent plants.

SUMMARY AND RECOMMENDATIONS

Great potential for increasing nectar production through phenotypic selection exists. Enhanced attractiveness of flowering species to nectar-gathering insects may lead to improvements in pollination and, ultimately, elevated seed and fruit yields. In addition, improvements in honey production can be expected under appropriate environmental conditions (Shuel 1989). Owing to their relative constancy, comparison of nectar-sugar quantities or "sugar values" per flower are the best parameters to be compared between lines, rather than amounts of nectar present or its carbohydrate concentration (Beutler 1953, Shuel 1989).

Still modest are the number of studies devoted to inheritance of nectar production, and that have relied on structural features of flowers correlated with nectar characteristics. Accordingly, it is premature to make confident recommendations, until further research of this kind is performed. Future studies which focus on the following, promising structural features of flowers would help elucidate their value for selection: thick flower stalk (peduncle); large number of vascular bundles in the flower stalk; large cross-sectional area of functional phloem in the flower stalk; large "nectar reservoir" diameter in legume florets; large volume, height and diameter of the receptacle; large phloem supply to the nectary(ies); large size of the banner petal in legumes; and high constancy in lateral-nectary size within flowers of the Brassicaceae.

Rejection of accessions that possess flowers whose stalks are thin, or whose floral nectaries possess high numbers of stomata, is advisable for programmes geared at breeding for high nectar-sugar production. These traits may be added to non-structural features such as few flowers per plant, weak aroma, and florets with yellow-green corollas (alfalfa), which were recommended previously (Shuel 1989) to be avoided.

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Tables

Table 1. Length of gynoecium and total nectar sugar per flower of *Vicia faba* cv. *Aqua dulce* after 36 h in culture. Means {± S.E. (range)} followed by a different let-ter are significantly different ($P < 0.05$) (Davis and Gunning 1991, Davis 1992)

Selection	n	Bean length (mm)	n	Nectar sugar per flower (µg)
A	15	18.79^{ab} ± 0.09 (18.25-19.3)	16	63.11^{a} ± 14.25 (10.01-217.01)
B	14	18.49^{b} ± 0.15 (17.4-19.4)	14	184.09^{bc} ± 42.66 (21.49-486.74)
D	15	18.59^{ab} ± 0.14 (17.3-19.4)	15	139.03^{b} ± 33.50 (15.75-462.95)
E	13	18.72^{ab} ± 0.14 (17.95-19.8)	13	114.83^{b} ± 16.53 (40.56-207.35)
K	15	18.95^{a} ± 0.12 (18.2-19.6)	15	275.51^{c} ± 40.66 (58.13-704.02)

Table 2. Correlation coefficients between nectar volume and diameter of the floret receptacle from varieties of *Medicago sativa* based on individual plants and plot means, and among selected subpopulations (data from Teuber et al. 1983).

	Variety		a
Method of correlation	*CUF 101*	*Team*	*Vernal*
Individual plants	0.282 (P = 0.01)	0.359 (P = 0.01)	0.302 (P = 0.01)
Plot means	0.235 (P = 0.05)	0.269 (P = 0.05)	0.347 (P = 0.01)
Among plots within subpopulations selected for			
- Nectar volume	0.509 (P = 0.05)	0.249 (ns)	0.721 (P = 0.01)
- Receptacle diameter	0.233 (ns)	0.405 (ns)	0.371 (ns)

Table 3. Area of nectary surface, density of nectary stomata and quantity of nectar sugar per flower produced by hypanthial nectaries (data from Weryszko-Chmielewska et al. 1997 and Davis 1997, respectively).

Nectary Family	No. stomata Species	Nectar sugar per surface (mm^2)	per mm^2	bagged flower (mg)
Rosaceae	*Aronia melanocarpa*	0.5192	126	1.58
	Chaenomeles japonica	0.9526	10	0.48
	Chaenomeles speciosa	1.4136	6	1.78
	Cydonia oblonga	2.2035	38	0.73
Myrtaceae	*Eucalyptus cosmophylla*	- - -	348 ± 15	18.49 ± 1.45
	Eucalyptus grandis	- - -	299 ± 10	3.32 ± 1.03
	Eucalyptus pulverulenta	- - -	163 ± 7	15.18 ± 7.74

Table 4 – Nectar volume and number of nectary stomata per floret in clones of alfalfa (*Medicago sativa*) (data from Teuber et al. 1980).

Cultivar	Clone no.	Nectar volume per 100 florets (µL)	Number of nectary stomata
Vernal	271	128.9	15.0 ± 0.6
	324	59.8	9.7 ± 0.6
	290	16.2	24.7 ± 1.9
Team	475	95.0	17.7 ± 0.5
	454	80.9	12.0 ± 0.5
	460	25.6	6.8 ± 0.5

Figures

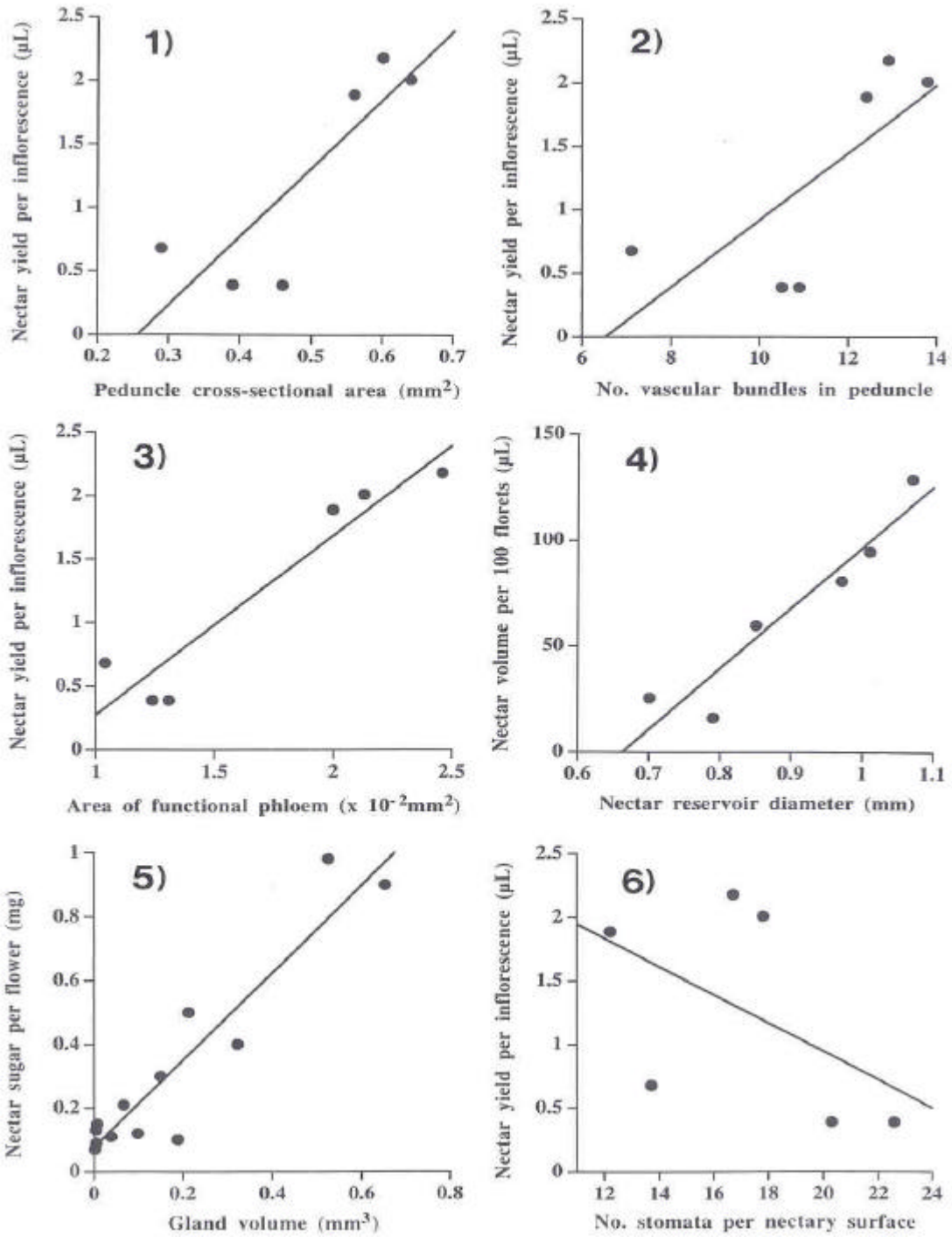

Fig. 1. *Lotus corniculatus* ($r = 0.856$, $P < 0.05$; $Y = 5.386X - 1.383$) (Murrell et al. 1982a).
Fig. 2. *Lotus corniculatus* ($r = 0.738$, $0.10 > P > 0.05$; $Y = 0.264X - 1.721$) (Murrell et al. 1982a).
Fig. 3. *Lotus corniculatus* ($r = 0.950$, $P < 0.01$; $Y = 1.412X - 1.140$) (Murrell et al. 1982a).
Fig. 4. *Medicago sativa* ($r = 0.950$, $P < 0.005$;$Y = 286.73X - 189.84$) (Teuber et al. 1980).
Fig. 5. Thirteen legume species ($r = 0.935$, $P < 0.05$; $Y = 0.07 + 1.3737X$) (Gulyás and Kincsek 1982).
Fig. 6. *Lotus corniculatus* ($r = -0.509$, $P < 0.3$; $Y = 3.172 - 0.111X$) (Murrell et al. 1982a).

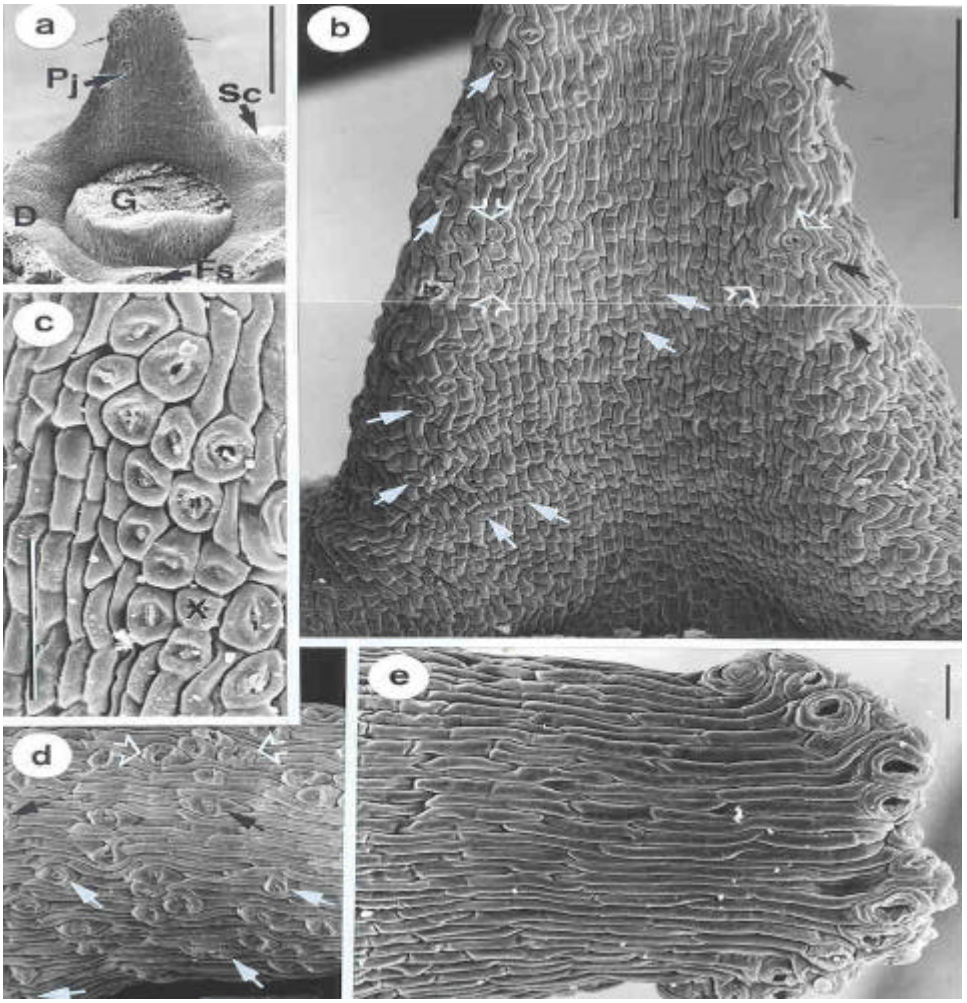

Fig. 7. Scanning electron micrographs of nectaries of field-collected or cultured, mature flowers of *Vicia faba* L. cv. *Aquadulce*. All flowers contained nectar when fixed for microscopy.

a) Dissected floral nectary from flower K1 sampled directly from a field plot. The nectary consists of a projection (Pj) opposite the flower's free stamen (Fs), plus a disk (D) that surrounds the base of the removed gynoecium (G). Base of staminal column (Sc). Note modified stomata (arrows) predominantly at projection tip. Scale bar = 500 µm.

b) Basal 5/8ths of the gynoecial face of nectary projection of flower A3 taken from the field. Approx. 20% of the modified stomata are indicated by solid arrows; most stomata are still immature and evident along the entire length of the projection. Two chains of four adjacent stomata are indicated between open arrows. Scale bar = 200 µm.

c) Left side at mid-region of the gynoecial face of projection of flower A1 sampled from the field, illustrating 16 of an aggregate of 17 adjacent, predominantly-immature modified stomata among elongate epidermal cells. One stomate (X) is practically surrounded by guard cells of five other stomata. Scale bar = 100 µm.

d) Mid-region along the staminal face of projection of cultured flower A21. Approx. 20% of the modified stomata, mostly immature, are indicated by solid arrows. Note cluster of four stomata that are connected (open arrows). Projection tip to the right. Scale bar = 100 µm.

e) Apex of the staminal face of projection of cultured flower K12. Below tip region of projection, note absence of modified stomata among elongate epidermal cells. Scale bar = 50 µm.