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## HERITABILITY OF NECTAR TRAITS: WHY DO WE KNOW SO LITTLE?

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**Abstract.** Although floral and extrafloral nectar traits are important for plant reproduction and defense, we know little about their genetic basis. Only a handful of studies have quantified heritable variation for nectar traits, primarily in controlled environments that minimize environmental variation. Most such studies have reported strong genetic influences, with heritabilities often  $>0.35$ . However, because nectar traits are often very responsive to environmental variation, even substantial amounts of genetic variation may be swamped out in the field. Environmental variation deserves to be studied in its own right, including exploration of genotype  $\times$  environment interaction for nectar traits. Most genetic studies of nectar have focused on production rate and concentration, whereas we know almost nothing about the heritability of other important traits such as production patterns, sugar ratios, amino acid composition, taste, and scent. Likewise, almost nothing is known about the heritability of extrafloral nectar traits. Important progress on all of these fronts can be made using simple experimental designs to quantify environmental effects, genotype  $\times$  environment interactions, clonal repeatability, and correlated traits. There is great promise in molecular approaches, but their use will not obviate the need for more quantitative genetic studies in the field and greenhouse.

**Key words:** *environmental variation; extrafloral nectary; floral traits; heritability; nectar; nectar traits; quantitative genetics; sugar concentration.*

### INTRODUCTION

Nectaries are plant glands that secrete water, sugars, amino acids, and other chemicals (Fahn 2000). Because nectar is a valuable resource for many animals, nectaries have important ecological functions, and their attributes may affect community structure of both animals and plants (e.g., Feinsinger 1978, Brown and Kodric-Brown 1979, Blüthgen et al. 2000, Apple and Feener 2001, Neuhauser et al. 2003, Whitham et al. 2003; see other papers in this Special Feature).

Nectaries occur on many plant parts. Those on or in flowers are termed floral nectaries (Schmid 1988), and are thought to encourage visitation by potential pollinators. Studies of the adaptive significance of floral nectar traits such as nectar production rate are consistent with this view (e.g., Pyke et al. 1988, Creswell and Galen 1991, Real and Rathcke 1991, Mitchell 1993, Hodges 1995, Meléndez-Ackerman et al. 1997, Mitchell et al. 1998). The adaptive nature of other nectar traits (e.g., chemical composition, taste, temporal patterns of nectar secretion) is often hypothesized (Faegri and van der Pijl 1971, Adler 2000, Gardener and Gillman 2002; see Waser 1983), but seldom tested.

Nectaries on leaves, stems, and other nonfloral plant structures are termed extrafloral nectaries. Tests generally confirm the hypothesis that extrafloral nectaries attract predatory insects such as ants that reduce her-

bivore activity and increase plant success (reviewed in Bentley 1977, Beattie 1985, Koptur 1992). Other adaptive hypotheses include distraction of ants from floral nectaries (Wagner and Kay 2002) and attracting prey to carnivorous plants (Cresswell 1993).

These hypothesized adaptive functions imply that nectary attributes have evolved in response to natural selection. Phenotypic selection on nectar traits is indeed frequent (see e.g., Bentley 1977, Beattie 1985, Pyke et al. 1988, Creswell and Galen 1991, Real and Rathcke 1991, Koptur 1992, Creswell 1993, Mitchell 1993, Hodges 1995, Meléndez-Ackerman et al. 1977, Mitchell et al. 1998, Wagner and Kay 2002), but for it to cause evolutionary change, nectar traits must be heritable (or must have been heritable in the past), and genetic correlations with other selected traits must not prevent evolutionary response to selection (Lande and Arnold 1983, Falconer 1989). However, little is known about the genetic basis of nectar traits, leaving many of these arguments merely speculative. Here I review what little is known about the genetics of nectar traits, suggest reasons for the dearth of knowledge, and highlight promising approaches for the future.

### ESTIMATING HERITABILITY OF NECTAR TRAITS

The most critical information for predicting response to selection is the additive genetic variance ( $V_A$ ), from which one can calculate narrow sense heritability ( $h^2 = V_A/V_P$ , where  $V_P$  is total phenotypic variance; Falconer 1989). Heritability determines the opportunity for response in the short term; if  $h^2$  is small, the immediate response to selection will be limited, whereas

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TABLE 1. Published reports of heritability of nectar traits in wild plant species, all involving floral nectar.

Study	Species	Method†	Environment	Heritabilities			
				Volume‡	Sugar concentration§	Total sugar	Diurnal pattern¶
Mitchell and Shaw (1993)	<i>Penstemon centranthifolius</i>	crosses	lathhouse	0.38*	0.00	0.37*	...
Mitchell and Shaw (1993)	<i>Penstemon centranthifolius</i>	clones	lathhouse	0.53*	0.04	0.47*	...
Campbell (1996)	<i>Ipomopsis aggregata</i>	crosses	field	0.05–0.15	...	0.00–0.15	...
Boose (1997)	<i>Epilobium canum</i>	clones	greenhouse	0.64*	...	...	*
Klinkhamer and van der Veen-van Wijk (1999)	<i>Echium vulgare</i>	clones	growth chamber	0.48*	0.62*	0.62*	...
Vogler et al. (1999)	<i>Campanula punctuloides</i>	clones	growth chamber	0.24*	...	...	...
Worley and Barrett (2000)	<i>Eichornia paniculata</i>	correlated response	greenhouse	*	...	...	...

Note: Ellipses indicate that no data are available.

\*  $P < 0.05$ .

† The different methods are explained in *Estimating heritability of nectar traits*; in the table, “crosses” indicates those studies that used breeding designs (paternal half sib or diallel designs) to estimate components of variation. All studies except Campbell (1996) involved potted plants.

‡ The volume of nectar secreted in a given time period (usually 24 h).

§ Mass/total mass of sugars, measured by a refractometer.

|| Sucrose equivalents (mg) secreted in some known period of time (calculated from volume and concentration; see Kearns and Inouye 1993).

¶ Changes in secretion rate or concentration over a 24-h period.

large  $h^2$  allows a strong response, assuming no constraining genetic correlations.

One approach to estimating  $V_A$  and  $h^2$  is to cross individuals under one of several standard breeding designs, then compare phenotypes among offspring, or between offspring and parents. Because the genetic relationships among individuals are known, one can interpret the similarity of kin in terms of genetic components of variation, including  $V_A$  (Falconer 1989, Lynch and Walsh 1998).

A second approach is to select on nectar traits and measure the direct response to selection (e.g., Teuber et al. 1990). Conversely, one may select on a non-nectar trait and assess the correlated response in nectar; a significant change in the nectar trait should indicate that it is heritable, and genetically correlated with the selected trait. However, correlated response to selection is often more variable than is direct response, making interpretation of such studies difficult (Gromko 1995).

Although the methods just described can give clean estimates of heritability, they are very demanding of time and resources, because reliable heritability measurements require evaluation of many hundreds of individuals of known genetic relationship. For organisms like plants, which can be clonally replicated, there is a less rigorous but much easier way to get an indication of overall genetic variation. By raising several ramets of several different genetic individuals, one can use nested ANOVA to partition the variance in nectar traits into within- and between-genet components (Falconer 1989). Thus, differences among genets estimate  $V_G$  (the

total genetic variance, which is the sum of  $V_A$  and other genetic sources of variance; this estimate also includes maternal effects). The fraction of total variance that is genetically based is referred to as the broad-sense heritability ( $H^2 = V_G/V_P$ ), or clonal repeatability. Broad-sense heritability therefore is an indirect index of  $V_A$  (the genetic variation that contributes to response to selection), and provides an upper bound on narrow-sense heritability. Thus, although studies of  $H^2$  are often more tractable than are studies of  $h^2$ , they are likely to overestimate evolutionary potential, and so must be interpreted with caution.

#### CURRENT KNOWLEDGE OF THE GENETICS OF NECTAR TRAITS

It is straightforward to summarize knowledge of the heritability of nectar traits for wild plants because there are, to my knowledge, only seven studies to date (Table 1). Four studies estimated  $V_G$  and  $H^2$  using clonal replication in controlled environments (Mitchell and Shaw 1993, Boose 1997, Klinkhamer and van der Veen-van Wijk 1999, Vogler et al. 1999). Two used breeding designs to estimate  $V_A$  and  $h^2$ ; one in a controlled environment (Mitchell and Shaw 1993; *Penstemon*), and one in the field (Campbell 1996; *Ipomopsis*). Finally, one selected on *Eichornia* flower number and size in a greenhouse for two generations, and found a significant correlated response in nectar production, implying substantial heritability (Worley and Barrett 2000). There is slightly more information available for cul-

tivated crops (see Rathcke 1992), with similar conclusions.

These few studies provide several important insights into the genetics of nectar traits.

First, there is abundant genetic variation. For most nectar traits,  $V_A$  or  $V_G$  was large, and  $h^2$  or  $H^2$  was often  $>0.35$  (non-nectar floral traits generally show similar to slightly higher heritabilities; e.g., Mitchell and Shaw 1993, Campbell 1996). This suggests that there is substantial opportunity for a response to selection on these traits, but also begs the question of what maintains that variation.

Second, non-genetic (mostly environmental) variation in nectar traits was large in all studies, even in controlled environments. Notably, the only field study (Campbell 1996) found that substantial  $V_A$  was swamped by the enormous environmental variation in *Ipomopsis* nectar production rate that occurs in the field.

Third, only a limited suite of nectar traits have been considered; primarily nectar production rate. For the most part, heritabilities of other important traits such as nectar chemistry (e.g., concentration of sugars, amino acids, toxins), taste, scent, age effects, response to nectar removal, temporal patterns, etc., have not been investigated. These traits are probably less plastic in response to environmental variation than is production rate (e.g., Pleasants 1983, Mitchell and Shaw 1993), and so might exhibit higher heritabilities.

Fourth, I know of no studies on the quantitative genetic basis of extrafloral nectar traits. However, there is a small literature on heritability of extrafloral nectary morphology; Rudgers (2004) found significant heritability of extrafloral nectary size for *Gossypium thurberi* (and demonstrated that larger nectaries have higher nectar production rates), and the absence of extrafloral nectaries is inherited as a single-locus trait in some crops (e.g., Rhyne 1969).

Fifth, genetic correlations between nectar traits and other floral and vegetative traits are rarely estimated (but see Mitchell and Shaw 1993, Campbell 1996, Boose 1997, Adler 2000, Worley and Barrett 2000). Such correlations may act as constraints on response to selection (Mitchell et al. 1998), and may conceivably contribute to the trait associations that correspond to floral syndromes (Wilson and Thomson 1996).

#### WHY ARE THERE SO FEW DATA ON HERITABILITY OF NECTAR TRAITS?

Given that nectar traits are a primary focus of so much speculation about adaptation, why are there so few data on their heritability? Probably one of the main reasons is that nectar traits can be difficult to measure. Some of the difficulties in measuring nectar traits include the following.

- 1) Nectar trait expression changes over time: over the course of a day, a flower's life span, a season,

and years (e.g., Pleasants 1983, Devlin et al. 1987, Real and Rathcke 1991, Hodges 1993, Jakobsen and Kristjansson 1994).

- 2) Nectar trait expression is labile in response to the biotic environment. For example, herbivory or nectar removal can affect nectar production rate and composition (e.g., Agrawal and Rutter 1998, Krupnick et al. 1999, Castellanos et al. 2002), and large plants may produce more nectar per flower than small plants (Pleasants and Chaplin 1983, Harder and Cruzan 1990).
- 3) Nectar traits are highly plastic in response to the abiotic environment. Light, water, fertilization, temperature,  $\text{CO}_2$  concentration, and a host of other factors can strongly affect nectar (e.g., references in Zimmerman 1988, Smith et al. 1990, Carroll et al. 2001, Gardener and Gillman 2001).
- 4) Nectar traits (especially secretion rate) can vary greatly among flowers within plants (e.g., Feinsinger 1983, Heil et al. 2000, Gardener and Gillman 2001). This increases the importance of care and skill in nectar sampling, and requires larger sample sizes, further slowing the work.
- 5) Nectar trait measurements are not as simple as those for many other traits (for methods, see Kearns and Inouye [1993]). Some reasons include the following: a) *They require extra preparation*, including shielding nectaries from visitation several hours before sampling, sampling at a consistent time after nectaries are drained, and dealing with intrinsic lability and time dependency in nectar traits (e.g., flower age, nectar resorption). b) *They take time*. Beyond the preparation mentioned earlier, nectar samples often take over twice as long to collect and process as do traits like flower or leaf size (*personal observation*). c) *They take skill and practice*. For example, removing all nectar requires thorough probing, which can damage nectaries or contaminate samples if done improperly. d) *They can require specialized and expensive equipment and training* (e.g., chemical analyses; Baker and Baker 1983).

For these and other reasons, the study of nectar traits may seem beyond the capacity of many researchers, especially given that they often are assessing heritabilities of many other traits at the same time.

Because studies of heritability (especially using breeding designs) require huge numbers of individuals, it is perhaps understandable that so little is known about the heritability of nectar. However, because of the important role of nectar traits in plant and animal ecology, and the prevalence of studies on their adaptive nature, such work would be valuable. Fortunately, most of the issues that I have raised can be addressed to at least some extent by hard work, ingenuity (e.g., Castellanos et al. 2002), and collaboration. Note that graduate students (and their collaborators) led most of the studies

in Table 1, indicating that motivated individuals can make good progress with minimal resources.

#### SUGGESTIONS FOR FUTURE WORK

*First, more work is needed on more traits.*—The accumulating work on current selection generates a pressing demand for more studies on the heritability of nectar traits, in a wider variety of species. It is important to move beyond a strictly energetic focus (volume and concentration), and to make progress on other features of putative adaptive significance. Nectar traits that play an important role in current models of trait evolution deserve extra attention (e.g., pollen presentation theory suggests the importance of dynamic adjustment of nectar production rate to maintain a constant standing crop; Thomson et al. 2000, Castellanos et al. 2002).

A tractable first step in documenting heritability of nectar traits is to use clonal repeatability (or even open-pollinated maternal sibships) to provide an index of genetic influences (see Mitchell and Shaw 1993, Havens 1994). Although such designs provide only a rough idea of narrow-sense heritability and genetic correlations (but see Mitchell and Shaw 1993), they are a start, and may be all that is feasible for difficult-to-measure traits such as nectar chemistry or dynamic regulation of nectar volume. They also can be used to improve understanding of non-genetic sources of variation in nectar traits (see *Embrace the variation*). If more detail is required, one can then move on to breeding designs (e.g., paternal half sibs) with a better starting knowledge of the expected variation and necessary sample size.

Molecular approaches may also be useful for understanding genetic influences on nectar traits, although such work requires a strong understanding of their quantitative genetic basis (Lynch and Walsh 1998). Studies of quantitative trait loci (QTL) have already identified chromosomal regions related to nectar trait differences among species of *Mimulus* (Bradshaw et al. 1998; see also Hodges et al. 2002). It is as yet unknown whether the same regions are involved in intraspecific variation. More generally, it would be interesting to know if these QTL regions contain any of the several genes currently implicated in nectary structure and function (e.g., Nelson et al. 1997, Bowman and Smyth 1999, Ge et al. 2000, Song et al. 2000, Baum et al. 2001, Carter and Thornburg 2002). Identification and study of nectar-specific genes and proteins is still in its infancy, but has the potential to open up many useful new opportunities (see Tatar 2000), including genetic manipulation of nectar phenotypes for field experiments, direct screening of field plants for alleles that alter nectar trait expression, and determination of the extent to which nectar traits are likely to be correlated with other traits at the gene level. Further progress on molecular characterization of the genetic influences on nectar production will be valuable. How-

ever, experience with other traits (such as petal color; e.g., Glover and Martin 1998, Clegg and Durbin 2000, Irwin et al. 2003; see also Tyner et al. 2002) indicates that the effects of specific genes on labile physiological traits such as nectar will not be simple. Instead there may be complicated interactions among genes, proteins, and the environment that produce unexpected consequences and genetic correlations (e.g., Gavin et al. 2002). Furthermore, nectaries probably evolved many times independently (e.g., Smets et al. 2000), so that distantly related species probably have divergent genetic controls on nectar traits. There is great promise in molecular approaches once they mature, but their use will not obviate the need for true quantitative genetic studies in the field and greenhouse.

*Second, embrace the variation.*—The great variation among and within plants in nectar traits is a reality faced by nectarivores. We need a much better understanding of the causes and consequences of such variation. Three types of variability deserve particular attention.

1. *Environmental variability in the field.*—There is a large literature concerning environmental effects on, and plasticity of, nectar traits, but mostly in controlled environments. Such work is necessary for understanding the causes of variation in expression, and should be extended, especially in new areas such as global change (e.g., Rusterholz and Erhardt 1998, Davis 2003). However, understanding the sources and consequences of phenotypic variation and selection in the wild requires more fieldwork (Campbell 1996, Herrera 1996), including studies of variation within years (e.g., Campbell and Halama 1993) and between years (e.g., Real and Rathcke 1991). Stratton's (1994) studies of non-nectar traits exemplify one potentially useful approach. He planted the same three genotypes of *Eriogonum annuus* together in many microsites in an old field, and recorded phenotypic responses. A nectar-focused version of such an experiment could be very informative to field biologists interested in sources of variation, patterns of natural selection, and genotype  $\times$  environment interactions.

2. *Genotype  $\times$  environment interaction.*—The strong dependence of nectar traits on environmental conditions (including both biotic and abiotic environments) might be genotype-specific. Such heritable differences in plasticity are known as genotype  $\times$  environment (G $\times$ E) interactions (Falconer 1989). If the rank order of genotypes for nectar traits changes across environments, there may be especially important consequences for the rate and direction of trait evolution, potentially promoting evolution of adaptive plasticity and maintenance of genetic variation (e.g., Via and Lande 1985, Mazer and Schick 1991, Pigliucci 2001). For example, if the genotype producing the most nectar in dry years produces the least in wet years, the response to directional selection would be slowed, and  $V_A$  would be maintained.

To date only three studies have tested for nectar trait  $G \times E$  interactions (Campbell 1996, Boose 1997, Vogler et al. 1999). All found significant  $G \times E$  interactions for nectar production rate, whereas Campbell (1996) found no significant  $G \times E$  interactions for nectar concentration. I know of no other studies on this topic (but see Devlin 1988), despite the large literature on environmental effects. Important unanswered questions include the following. How common, strong, or adaptive are  $G \times E$  interactions? To what extent do traits differ in  $G \times E$  interactions, and why? Does the magnitude of  $G \times E$  interactions differ across types of environmental variation (e.g., light vs. water vs. herbivory), and how does this relate to variation experienced in the field? Once  $G \times E$  interactions are well documented for these traits, molecular analyses may be helpful in determining which nectar genes are active in different environments.

In the context of this paper,  $G \times E$  interaction is potentially the most important and under-explored area of nectar research. Paradoxically, it is relatively straightforward to assess using clone or maternal sibship analysis.

3. *Within-plant phenotypic variability.*—There is often substantial variation in nectar traits (especially production rate) among nectaries on a plant, even when nectary age and other factors are controlled. Although there is always the possibility that this variation is a sampling artifact (e.g., ineffectively bagged nectaries, or difficulty in removing all nectar), careful studies continue to document this pattern (e.g., Feinsinger 1978, 1983, Freeman and Wilken 1987, Klinkhamer and van der Veen-van Wijk 1999).

What is the significance of highly variable nectar traits? In part, this variation certainly stems from developmental and environmental noise, so further study of these issues is vital. However, several researchers have argued that variability (at least for floral nectar production rate) may itself be adaptive (e.g., Feinsinger 1978, 1983, Pleasants 1983, Rathcke 1992) because pollinators encountering variable rewards are more likely to move to another plant, promoting outcrossing. For example, one might predict that floral nectaries should exhibit more variable nectar production rates than extrafloral nectaries on the same plant (after accounting for age-related variation in production rates), because variability in the rewards from extrafloral nectaries could reduce within-plant movement of ants, with the consequence that the plant is less well defended. This prediction is readily testable, but has not been investigated (see Koptur 1994). Furthermore, no studies have quantified the heritability of between-flower variation in nectar production rates. From the pollinator point of view, experimental manipulation of resource variability and distribution, and observation of how this affects visitor behavior is also a promising approach (e.g., Biernaskie et al. 2002).

## CONCLUSION

The current lack of information on the genetic basis of nectar traits is remarkable given the many adaptive stories told about them. In concert with more studies of ongoing selection on nectar traits, and on their role in plant and animal communities, researchers should strive to document their genetic basis. As previously described, substantial progress can be made with moderate effort and expense. Indeed, in an academic semester, undergraduates could make good progress on simple clonal repeatability or  $G \times E$  studies. Knowledge of the genetic basis of nectar traits is necessary to improve our understanding of their adaptive nature, and the potential for evolutionary change.

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## LITERATURE CITED

- Adler, L. S. 2000. The ecological significance of toxic nectar. *Oikos* **91**:409–420.
- Agrawal, A. A., and M. T. Rutter. 1998. Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* **83**:227–236.
- Apple, J. L., and D. H. J. Feener. 2001. Ant visitation of extrafloral nectaries of *Passiflora*: the effects of nectary attributes and ant behavior on patterns in facultative ant-plant mutualisms. *Oecologia* **127**:409–416.
- Baker, H. G., and I. Baker. 1983. Floral nectar sugar constituents in relation to pollinator type. Pages 117–141 in C. E. Jones and J. R. Little, editors. *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, New York, USA.
- Baum, S. F., Y. Eshed, and J. L. Bowman. 2001. The *Arabidopsis* nectary is an ABC-independent floral structure. *Development* **128**:4657–4667.
- Beattie, A. J. 1985. *The evolutionary ecology of ant-plant mutualisms*. Cambridge University Press, New York, New York, USA.
- Bentley, B. L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics* **8**:407–427.
- Biernaskie, J. M., R. V. Cartar, and T. A. Hurly. 2002. Risk-averse inflorescence departure in hummingbirds and bumble bees: could plants benefit from variable nectar volumes? *Oikos* **98**:98–104.
- Blüthgen, N., M. Verhaagh, W. Goitia, K. Jaffe, W. Morawetz, and W. Barthlott. 2000. How plants shape the ant community in the Amazonian rainforest canopy: the key role of extrafloral nectaries and homopteran honeydew. *Oecologia* **125**:229–240.
- Boose, D. L. 1997. Sources of variation in floral nectar production rate in *Epilobium canum* (Onagraceae): implications for natural selection. *Oecologia* **110**:493–500.
- Bowman, J. L., and D. R. Smyth. 1999. CRABS CLAW, a gene that regulates carpel and nectar development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* **126**:2387–2396.
- Bradshaw, H. D. J., K. G. Otto, B. E. Frewen, J. K. McKay, and D. W. Schemske. 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). *Genetics* **149**:367–382.

- Brown, J. H., and A. Kodric-Brown. 1979. Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. *Ecology* **60**:1022–1035.
- Campbell, D. R. 1996. Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* **50**:1442–1453.
- Campbell, D. R., and D. R. Halama. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* **74**:1043–1051.
- Carroll, A. B., S. G. Pallardy, and C. Galen. 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany* **88**:438–446.
- Carter, C., and R. W. Thornburg. 2002. The nectary-specific pattern of expression of the tobacco Nectarin I promoter is regulated by multiple promoter elements. *Plant Molecular Biology* **51**:451–457.
- Castellanos, M. C., P. Wilson, and J. D. Thomson. 2002. Dynamic nectar replenishment in flowers of *Penstemon* (Scrophulariaceae). *American Journal of Botany* **89**:111–118.
- Clegg, M. T., and M. L. Durbin. 2000. Flower color variation: a model for the experimental study of evolution. *Proceedings of the National Academy of Sciences (USA)* **97**:7106–7023.
- Cresswell, J. E. 1993. The morphological correlates of prey capture and resource parasitism in pitchers of the carnivorous plant *Sarracenia purpurea*. *American Midland Naturalist* **129**:35–41.
- Cresswell, J. E., and C. Galen. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations—the pollination of *Polemonium viscosum*. *American Naturalist* **138**:1342–1353.
- Davis, A. R. 2003. Influence of elevated CO<sub>2</sub> and ultraviolet-B radiation levels on floral nectar production: a nectary-morphological perspective. *Plant Systematics and Evolution* **238**:169–181.
- Devlin, B. 1988. The effects of stress on reproductive characters of *Lobelia cardinalis*. *Ecology* **69**:1716–1720.
- Devlin, B., J. B. Horton, and A. G. Stephenson. 1987. Patterns of nectar production of *Lobelia cardinalis*. *American Midland Naturalist* **117**:289–295.
- Faegri, K., and L. van der Pijl. 1971. The principles of pollination ecology. Second edition. Pergamon, Oxford, UK.
- Fahn, A. 2000. Structure and function of secretory cells. *Advances in Botanical Research* **31**:37–75.
- Falconer, D. S. 1989. Introduction to quantitative genetics. Third edition. Longman, London, UK.
- Feinsinger, P. 1978. Ecological interactions between plants and hummingbirds in a successional tropical community. *Ecological Monographs* **48**:269–287.
- Feinsinger, P. 1983. Variable nectar secretion in a *Heliconia* species pollinated by hermit hummingbirds. *Biotropica* **15**:48–52.
- Freeman, C. E., and D. H. Wilken. 1987. Variation in nectar-sugar composition at the intra-plant level in *Ipomopsis longiflora* (Polemoniaceae). *American Journal of Botany* **74**:1681–1689.
- Gardener, M. C., and M. P. Gillman. 2001. Analyzing variability in nectar amino acids: composition is less variable than concentration. *Journal of Chemical Ecology* **27**:2545–2558.
- Gardener, M. C., and M. P. Gillman. 2002. The taste of nectar—a neglected area of pollination ecology. *Oikos* **98**:552–557.
- Gavin, A.-C., et al. 2002. Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**:141–147.
- Ge, Y.-X., G. C. Angenent, P. E. Wittich, J. Peters, J. Franken, M. Busscher, L.-M. Zhang, E. Dahlhaus, M. M. Kater, G. J. Wullems, and T. Creemers-Molenaar. 2000. NEC1, a novel gene, highly expressed in nectary tissue of *Petunia hybrida*. *Plant Journal* **24**:725–734.
- Glover, B. J., and C. Martin. 1998. The role of petal cell shape and pigmentation in pollination success in *Antirrhinum majus*. *Heredity* **80**:778–784.
- Gromko, M. H. 1995. Unpredictability of correlated response to selection: pleiotropy and sampling interact. *Evolution* **49**:685–693.
- Harder, L. D., and M. B. Cruzan. 1990. An evaluation of the physiological and evolutionary influences of inflorescence size and flower depth on nectar production. *Functional Ecology* **4**:559–572.
- Havens, K. 1994. Clonal repeatability of in vitro pollen tube growth rates in *Oenothera organensis* (Onagraceae). *American Journal of Botany* **81**:161–165.
- Heil, M., B. Fiala, B. Baumann, and K. E. Linsenmair. 2000. Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Functional Ecology* **14**:749–757.
- Herrera, C. M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. Pages 65–87 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral biology*. Chapman and Hall, New York, New York, USA.
- Hodges, S. A. 1993. Consistent interplant variation in nectar characteristics of *Mirabilis multiflora*. *Ecology* **74**:542–548.
- Hodges, S. A. 1995. The influence of nectar production on hawkmoth behavior, self pollination, and seed production in *Mirabilis multiflora* (Nyctaginaceae). *American Journal of Botany* **82**:197–204.
- Hodges, S. A., J. B. Whittall, M. Fulton, and J. Y. Yang. 2002. Genetics of floral traits influencing reproductive isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *American Naturalist* **159**:S51–60.
- Irwin, R. E., S. Y. Strauss, S. Storz, A. Emerson, and G. Guibert. 2003. The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology* **84**:1733–1743.
- Jakobsen, H. B., and K. Kristjansson. 1994. Influence of temperature and floret age on nectar secretion in *Trifolium repens* L. *Annals of Botany* **74**:327–334.
- Kearns, C. A., and D. W. Inouye. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.
- Klinkhamer, P. G. L., and C. A. M. van der Veen-van Wijk. 1999. Genetic variation in floral traits of *Echium vulgare*. *Oikos* **85**:515–522.
- Koptur, S. 1992. Extrafloral nectar-mediated interactions between insects and plants. Pages 81–129 in E. Bernays, editor. *Insect-plant interactions*. Volume IV. CRC Press, Boca Raton, Florida, USA.
- Koptur, S. 1994. Floral and extrafloral nectars of Costa Rican *Inga* trees: a comparison of their constituents and composition. *Biotropica* **26**:276–284.
- Krupnick, G. A., A. E. Weis, and D. R. Campbell. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* **80**:125–134.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* **37**:1210–1226.
- Lynch, M., and B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer, Sunderland, Massachusetts, USA.
- Mazer, S. J., and C. T. Schick. 1991. Constancy of population parameters for life history and floral traits in *Raphanus sativus* L. I. Norms of reaction and the nature of genotype by environment interactions. *Heredity* **67**:143–156.
- Meléndez-Ackerman, E., D. R. Campbell, and N. M. Waser. 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. *Ecology* **78**:2532–2541.

- Mitchell, R. J. 1993. Adaptive significance of *Ipomopsis aggregata* nectar production—observation and experiment in the field. *Evolution* **47**:25–35.
- Mitchell, R. J., and R. G. Shaw. 1993. Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae)—clones and crosses. *Heredity* **71**:185–192.
- Mitchell, R. J., R. G. Shaw, and N. M. Waser. 1998. Pollinator selection, quantitative genetics, and predicted evolutionary responses of floral traits in *Penstemon centranthifolius* (Scrophulariaceae). *International Journal of Plant Sciences* **159**:331–337.
- Nelson, D. E., B. Glaunsinger, and H. J. Bohnert. 1997. Abundant accumulation of the calcium-binding molecular chaperone calreticulin in specific floral tissues of *Arabidopsis thaliana*. *Plant Physiology* **114**:29–37.
- Neuhauser, C., D. A. Andow, G. E. Heimpel, G. May, R. G. Shaw, and S. Wagenius. 2003. Community genetics: expanding the synthesis of ecology and genetics. *Ecology* **84**:545–558.
- Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Pleasants, J. M. 1983. Nectar production patterns in *Ipomopsis aggregata*. *American Journal of Botany* **70**:1468–1475.
- Pleasants, J. M., and S. J. Chaplin. 1983. Nectar production rates of *Asclepias quadrifolia*: causes and consequences of individual variation. *Oecologia* **69**:232–238.
- Pyke, G. H., L. P. Day, and R. A. Wale. 1988. Pollination ecology of christmas bells (*Blandfordia nobilis* Sm.)—effects of adding artificial nectar on pollen removal and seed set. *Australian Journal of Ecology* **13**:279–284.
- Rathcke, B. J. 1992. Nectar distributions, pollinator behavior, and plant reproductive success. Pages 113–138 in M. D. Hunter, T. Ohgushi, and P. W. Price, editors. Effects of resource distribution on animal–plant interactions. Academic Press, New York, New York, USA.
- Real, L. A., and B. J. Rathcke. 1991. Individual variation in nectar production and its effect on fitness in *Kalmia latifolia*. *Ecology* **72**:149–155.
- Rhyne, C. L. 1969. Inheritance of extra-floral nectaries in cotton. *Advancing Frontiers of Plant Sciences* **13**:121–135.
- Rudgers, J. A. 2004. Enemies of herbivores can shape plant traits: selection in a facultative ant–plant mutualism. *Ecology* **85**:1495–1502.
- Rusterholz, H. P., and A. Erhardt. 1998. Effects of elevated CO<sub>2</sub> on flowering phenology and nectar production of nectar plants important for butterflies of calcareous grasslands. *Oecologia* **113**:341–349.
- Schmid, R. 1988. Reproductive versus extra-reproductive nectaries—historical perspective and terminological recommendations. *Botanical Review* **54**:179–232.
- Smets, E. F., L.-P. Ronse Decraene, P. Caris, and P. J. Rudall. 2000. Floral nectaries in monocotyledons: distribution and evolution. Pages 230–240 in K. L. Wilson and D. A. Morrison, editors. Monocots: systematics and evolution. CSIRO, Melbourne, Australia.
- Smith, L. L., J. Lanza, and G. C. Smith. 1990. Amino acid concentrations in extrafloral nectar of *Impatiens sultani* increase after simulated herbivory. *Ecology* **71**:107–115.
- Song, J. T., H. S. Seo, S. I. Song, J. S. Lee, and Y. D. Choi. 2000. NTR1 encodes a floral nectary-specific gene in *Brassica campestris* L. ssp. *pekinensis*. *Plant Molecular Biology* **42**:647–655.
- Stratton, D. A. 1994. Genotype-by-environment interactions for fitness of *Erigeron annuus* show fine scale selective heterogeneity. *Evolution* **48**:1607–1618.
- Tatar, M. 2000. Transgenic organisms in evolutionary ecology. *Trends in Ecology and Evolution* **15**:207–211.
- Teuber, L. R., C. M. Rincker, and D. K. Barnes. 1990. Seed yield characteristics of alfalfa populations selected for receptacle diameter and nectar volume. *Crop Science* **30**:579–583.
- Thomson, J. D., P. Wilson, M. Valenzuela, and M. Malzone. 2000. Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biology* **15**:11–29.
- Tyner, S. D., et al. 2002. p53 mutant mice that display early aging-associated phenotypes. *Nature* **415**:45–53.
- Via, S., and R. Lande. 1985. Genotype–environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**:505–522.
- Vogler, D. W., S. Peretz, and A. G. Stephenson. 1999. Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment and ontogeny of *Campanula rapunculoides* (Campanulaceae). *American Journal of Botany* **86**:482–494.
- Wagner, D., and A. Kay. 2002. Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis. *Evolutionary Ecology Research* **4**:293–305.
- Waser, N. M. 1983. The adaptive nature of floral traits: ideas and evidence. Pages 241–285 in L. Real, editor. *Pollination Biology*. Academic Press, New York, New York, USA.
- Whitham, T. G., W. P. Young, G. D. Martinsen, C. A. Gehring, J. A. Schweitzer, S. M. Shuster, G. M. Wimp, D. G. Fischer, J. K. Bailey, R. L. Lindroth, S. Woolbright, and C. R. Kuske. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology* **84**:559–573.
- Wilson, P., and J. D. Thomson. 1996. How do flowers diverge? Pages 88–111 in D. G. Lloyd and S. C. H. Barrett, editors. *Floral Biology*. Chapman and Hall, New York, New York, USA.
- Worley, A. C., and S. C. H. Barrett. 2000. Evolution of floral display in *Eichornia paniculata* (Pontederiaceae): direct and correlated responses to selection on flower size and number. *Evolution* **54**:1533–1545.
- Zimmerman, M. 1988. Nectar production, flowering phenology, and strategies for pollination. Pages 157–178 in J. Lovett-Doust and L. Lovett-Doust, editors. *Plant Reproductive Ecology, Patterns and Strategies*. Oxford University Press, Oxford, UK.