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The sweetest thing: Advances in nectar research

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We all appreciate the beauty of flowers, but we seldom consider their function in the life cycle of the plant. The function of beautiful flowers is to advertise the presence of nectar. Floral nectar is the key component in the mutualism between flowering plants and their pollinators. Plants offer nectar as a reward for the transport of pollen by animal vectors. Studying nectar is challenging because of its complex physiology, complex polygenetic structure, and strong environmental variability. Recent advances set the stage for exciting future research that combines genetics and physiology to study ecological and evolutionary questions.

Introduction

Floral nectar is a key innovation of angiosperms that evolved as a reward to visitors that transport pollen in return. It is a sugar-rich fluid dominated by the hexoses glucose and fructose, and the disaccharide sucrose. Nectar allows flowers to 'outsource' the pollination business to animal vectors, which assure a directional, accurate, and efficient transfer of pollen compared to wind pollination. The establishment of animal-mediated pollination not only solves a problem but also creates new ones. First, nectar production is costly in terms of seed production and photoassimilate allocation [1,2]. Second, the sugar solution does not only attract pollinators. Nectar robbers and microbes may consume the reward without transferring pollen. Third, pollen may be deposited at the wrong recipient, that is, a different plant species. While this latter problem can be reduced with the evolution of more exclusive relationships with few or even only one pollinator species, plants using this strategy limit their potential distribution to the distribution of their pollinators, which may increase extinction risk (Figure 1).

Most floral traits are likely to be genetically complex, and few of the genes involved have been isolated so far. The identification of such genes will allow a genetic analysis of floral traits involved in plant–pollinator interactions. Downregulation of relevant genes can give information about the effect of single gene mutations on pollinator behavior [3,4,5••,6••]. Marker-assisted breeding (near isogenic lines) and transgenic plants can provide useful material for field assays [7••,8••].

We will briefly present the recent key advances in nectar research related to the following topics: first, the physiology of nectar sugar production; second, nectar composition, in particular the functions of primary and secondary compounds; and third, the genetics of nectar production. We will conclude with suggestions for important future research questions on nectar.

The physiology of nectar sugar production

The site of nectar production, secretion, and release are the nectaries (Figure 2). These specialized organs occur in or around vegetative or reproductive organs [9–11]. In evolutionary terms, the variability in location reflects the broad diversity of pollinators and their foraging behavior. The specification of nectaries does not depend on the ABC genes that control the specification of all other floral organs. This lack of genetic constraints may explain the flexibility in position [12].

Although nectaries may have active chloroplasts, carbohydrates for nectar production are mostly imported. Sucrose is transported from source tissues via the phloem and stored in the nectary parenchyma as starch [13,14]. Ren *et al.* [15] recently demonstrated in *Nicotiana* that starch-breakdown in nectary plastids not only produces nectar sugars but also causes an influx of sucrose into the nectaries. The expression of genes involved in starch synthesis and breakdown are tightly linked to nectary developmental stages, where starch catabolism is correlated with nectar release prior to anthesis [16].

Figure 1

Closely related species attract different pollinators. Left, *Petunia exserta* with Hylocharis chrysura; right, *P. axillaris* ssp. *axillaris* with hawkmoth *Manduca diffisa.* Nectar production is similar in the two species. Differences in color, fragrance, and architecture of the flower determine the specificity of the interaction. Photos: Alexandre Dell'Olivo.

Figure 2

Floral reward and floral display. Longitudinal section through a flower of *Petunia axillaris* ssp. *axillaris*. The nectaries (arrows) are concealed at the base of the gynoecium, favoring access to specific hawkmoths pollinators, and restricting access to unwanted visitors. Photos: Marc Grémillon.

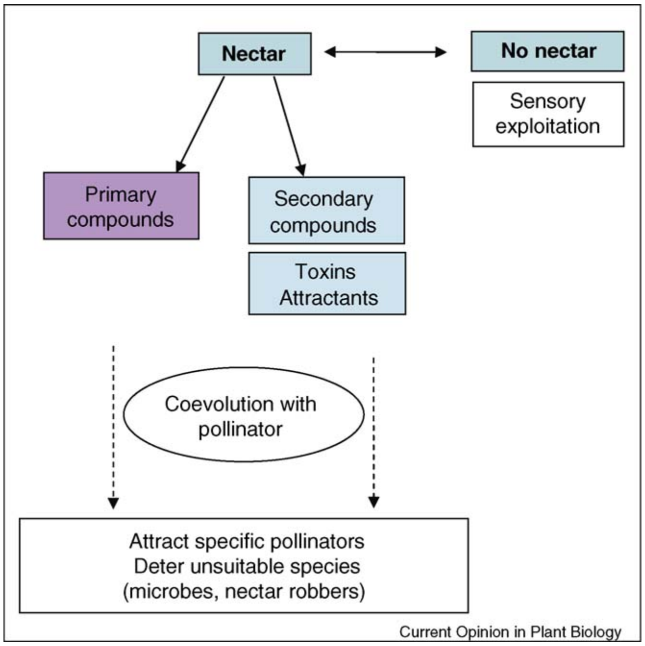
It was originally assumed that the production of glucose and fructose resulted from the hydrolysis of sucrose [17]. However, the ratio may deviate significantly from the expected 1:1 in many species. This discrepancy between theory and data was recently resolved [18••]: after the hydrolysis of sucrose, the hexoses are partially cycled through various biochemical pathways before being secreted into the lumen of the nectary. This more complex metabolism could explain a deviation from the 1:1 ratio. In addition, microbial degradation can alter nectar composition [19]. To counteract degradation and protect reproductive organs from microbial attack, some plants secrete antimicrobial hydrogen peroxide into the nectar [20].

Functions of nectar

From the plant's perspective, in an ideal scenario pollinators carry the maximum amount of pollen from one plant to the stigma of a conspecific while consuming minimal nectar (Figure 3). Limitation of nectar availability entices pollinators to forage on a larger number of flowers and enhance pollen distribution. Plants make a preselection by luring certain pollinator guilds via advertising floral traits like scent [21], petal pigmentation [22], and other floral structures (waxes, cell shape, etc.). Recently, Goyret *et al.* [23] demonstrated the importance of CO_2 emission as an attractant. *Datura wrightii* emits large amounts of CO_2 at anthesis when nectar volume is highest, provoking a strong attraction of the hawkmoth *Manduca sexta* toward the carbon dioxide source. Only insects with CO_2 sensing organs can receive this signal and choose the flowers with highest rewards. Species identity of the visitor and length and frequency of visits are thus crucial factors for plant reproductive success.

Both length and frequency of foraging bouts are regulated by the composition and concentration of primary and

Figure 3



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Functional relationship of nectar and floral visitors. Key strategic options how a plant may maximize its lifetime reproductive success by adjusting nectar quantity and composition. The first decision is whether to reward pollinators or to cheat through sensory exploitation of the pollinator's nervous system. In the case of nectar production, coevolution with preferred pollinators should lead to specific compositions of primary and secondary compounds that optimize visitation by pollinators help to reduce the number of unwanted visitors. Physiological and molecular approaches will play a major role in testing this evolutionary scenario.

secondary metabolites in the nectar. The long-standing dogma that pollinator preference is the driving selective force for nectar sugar composition [3] has been repeatedly supported [24–27]. Lotz and Schondube [25] provide an extreme case for the importance of sugar composition by demonstrating that two passerine bird clades cannot digest sucrose. In parallel, however, several authors recently provided evidence for the importance of sugar concentrations and nectar volume for pollinator preferences: for example, several species of birds consistently switched from a hexose preference in diluted nectars to a sucrose preference in a concentrated diet [28–30].

An important function of secondary compounds in the nectar is to repel less specialized or even illegitimate visitors such as nectar robbers and pathogens. However, secondary compounds may also regulate the duration of pollinator visits and as a consequence the number of plants visited. Irwin and Adler [5••] demonstrated that the occurrence of the alkaloid gelsemine in nectar of *Gelsemium sempervirens* significantly decreased both frequency and length of pollinator visitations but increased the number of flowers visited. A model demonstrates that under specific ecological conditions, plants can thus favorably influence pollen distribution patterns and promote outcrossing with alkaloids [5••]. Kessler and Baldwin [6••] found that nicotine in nectar repelled pollinators and decreased their visitation (drinking) times. In addition, they found that plants may counterbalance this effect with increasing amounts of the major volatile attractant, benzylacetone (BA). In subsequent field experiments, Kessler *et al.* [7••] utilized plants in which nicotine synthesis was knocked down, which resulted in an increased visiting time on fewer flowers. In contrast to that, transgenic plants with reduced BA emission received shorter visits on more flowers. Plants emitting both attractant and repellent produced more seeds than any of the manipulated experimental groups [7••]. Thus, complex blends of volatiles serve to optimize pollinator visitation and reduce visits by uninvited guests.

Some angiosperms, in particular orchid species, have evolved an alternative pollination strategy that involves no nectar production but still relies on pollinators (Figure 3). These species deceive their visitors by mimicking a mating partner or a rewarding species, often exaggerating attractiveness relative to models (for overviews see [31–33]). Sexually deceptive orchids, such as *Ophrys exaltata* fool their victims by producing female bee pheromones but actually in different relative proportions than found in bees. Apparently, the plant exploits a mating decision rule of male bees that makes them prefer novel pheromone combinations as an outbreeding strategy that promotes mating with immigrated females [34••]. With respect to food deceptive species, Peter and Johnson demonstrated that the mimic *Eulophia zeyheriana* differs in only 0.03 units in bee color space from its model, which implies according to bee vision studies [35••] that model and mimic are indistinguishable to the pollinator. Pollinators alter their flower visitation patterns if they encounter empty flowers: they switch plants faster and move larger distances between consecutive visits [36,37]. These changes actually provide some benefits to the mimic in the form of enlarged pollen dispersal radius and prevention of inbreeding [38,39]. Nevertheless, recent experiments on the deceptive orchid *Dactylorhiza sambucina* demonstrate that plants supplemented with nectar receive more visits and pollen [40••]. The authors conclude that the disadvantage of reduced visitation is outweighed by increasing the fitness advantage resulting from increased outbreeding.

Nectar genetics

Experimental manipulation of floral traits, such as supplementation/depletion of volatiles or sugars can give an indication of how these traits affect pollinator behavior and plant fitness. However, such experiments will rarely be conclusive. They do not account for the cost of production, and experiments are necessarily short-term. Nor give insight into the underlying molecular and genetic mechanisms. Designing plants with genetically modified nectars as seen in the studies discussed above offers obvious advantages [6••,7••]. The production of such genetic material is challenging, however. Characteristic for nectar is its substantial environmental variability in concentration, composition, and volume between populations [41], plants [42–44], also genders [45], and even interfloral and intrafloral variability from day to day [46,47].

Floral traits that affect pollinator behavior have the potential to lead to reproductive isolation. One of the most exciting aspects of plant reproductive biology is the fact that in many cases, plants with major phenotypic differences may be isolated in the wild but remain sexually compatible. A good example is the genus *Petunia* with species such as *P. axillaris*, *P. integrifolia*, and *P. exserta* that are partly or even completely reproductively isolated in their natural habitats, yet are routinely crossed in the laboratory. Controlled interspecific crosses make it possible to elucidate the genetic modifications underlying their contrasting pollination syndromes. Under controlled laboratory conditions, bee-pollinated *P. integrifolia* produces an average of 1.2 μl nectar, whereas in the moth-pollinated species *P. axillaris* it is as high as 13–23 μl [48,49••]. Such clear differences between sister species offer unique opportunities to study the genetic changes that have led to the evolution of new pollination syndromes and reproductive isolation. Four minor QTL (*vol* 4–7) were identified in an interspecific cross between the two *Petunia* species. The additive effect of *vol* 4–7 accounted for 30% of the difference between the parental lines [49••]. This suggests that nectar production is strongly polygenic. A different situation was found in *Mimulus*: half the phenotypic variance between two closely related species with a 80-fold difference in nectar volume could be explained by one single major QTL [50]. These few studies give first hints into the genetics of nectar traits. They demonstrate that, in addition to strong environmental variation, there is also abundant genetic variation and thus a substantial opportunity for a response to selection on these traits.

Conclusions and future directions

The field of nectar research has evolved in recent years. Advances in analytical methods have changed our views on the function of both the major and minor constituents. In particular, the unexpected chemical complexity of secondary metabolites in floral nectar translates into new insights into their ecological significance. An important field for future research concerns the role of individual traits that make up pollination syndromes. Can we untangle the specific function of nectar composition from other floral traits? Most of the experiments are conducted by conventional approaches such as nectar supplementation or depletion. Genetic manipulations in model organisms such as *Mimulus*, *Petunia*, and *Nicotiana* will be invaluable. What will be the effect of genetically reducing nectar content or composition? Will such cheating plants have reduced fitness because they are avoided by pollinators, or will fitness be increased due to enhanced outbreeding? We look forward to the answers to these and many other exciting questions.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

•• of outstanding interest

1. Southwick EE: **Photosynthate allocation to floral nectar — a neglected energy investment**. *Ecology* 1984, **65**:1775-1779.

2. Pyke GH: **What does it cost a plant to produce floral nectar?** *Nature* 1991, **350**:58-59.

3. Baker H, Baker I: **A brief historical review of the chemistry of floral nectar**. In *The Biology of Nectaries.* Edited by Bentley B, Elias T. Columbia University Press; 1983:127-152.

4. Liu F, Chen J, Chai J, Zhang X, Bai X, He D, Roubik DW: **Adaptive functions of defensive plant phenolics and a non-linear bee response to nectar components**. *Funct Ecol* 2007, **21**:96-100.

5. •• Irwin RE, Adler LS: **Nectar secondary compounds affect self-pollen transfer: implications for female and male reproduction**. *Ecology* 2008, **89**:2207-2217.

Secondary compounds are a common feature in floral nectars. They can repel undesirable floral visitors and control pollen movement patterns of pollinators. High nectar alkaloids causing an increased pollen export can benefit the plant.

6. •• Kessler D, Baldwin IT: **Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata***. *Plant J* 2007, **49**:840-854.

Secondary metabolites were identified in the headspace and nectar of glasshouse-grown and field-grown *Nicotiana attenuata* plants. Sixteen compounds were tested for attractiveness and repellence in two pollinator guilds and one nectar robber. Two of the substances can act as 'filters', and encourage or discourage floral visitors.

7. •• Kessler D, Gase K, Baldwin IT: **Field experiments with transformed plants reveal the sense of floral scents**. *Science* 2008, **321**:1200-1202.

Field assays with transgenic *Nicotiana* plants lacking the attractive compound benzyl acetone (BA), or repellent nicotine (N) or both were conducted to assess the impact of secondary compounds on pollinator behavior. BA enhances pollinator visits, N reduces drinking time; both are needed to maximize seed set and reproductive success.

8. •• Hoballah ME, Gubitz T, Stuurman J, Broger L, Barone M, Mandel T, Dell'Olivo A, Arnold M, Kuhlemeier C: **Single gene-mediated shift in pollinator attraction in *Petunia***. *Plant Cell* 2007, **19**:779-790.

Transcription factor AN2 regulates anthocyanin production in bee-pollinated *Petunia integrifolia*, but is inactivated in moth-pollinated *P. axillaris*. Transgenic introduction of active AN2 into *P. axillaris* partially restores petal color and causes a shift in pollinator visitation.

9. Wist TJ, Davis AR: **Floral structure and dynamics of nectar production in *Echinacea pallida* var. *angustifolia* (Asteraceae)**. *Int J Plant Sci* 2008, **169**:708-722.

10. Wist TJ, Davis AR: **Floral nectar production and nectary anatomy and ultrastructure of *Echinacea purpurea* (Asteraceae)**. *Ann Bot* 2006, **97**:177-193.

11. Nepi M, Stpiczynska M: **The complexity of nectar: secretion and resorption dynamically regulate nectar features**. *Naturwissenschaften* 2008, **95**:177-184.

12. Baum SF, Eshed Y, Bowman JL: **The *Arabidopsis* nectary is an ABC-independent floral structure**. *Development* 2001, **128**:4657-4667.

13. De la Barrera E, Nobel PS: **Nectar: properties, floral aspects, and speculations on origin**. *Trends Plant Sci* 2004, **9**:65-69.

14. Cawoy V, Kinet JM, Jacquemart AL: **Morphology of nectaries and biology of nectar production in the distylous species *Fagopyrum esculentum***. *Ann Bot* 2008, **102**:675-684.

15. Ren G, Healy RA, Klyne AM, Horner HT, James MG, Thornburg RW: **Transient starch metabolism in ornamental tobacco floral nectaries regulates nectar composition and release**. *Plant Sci* 2007, **173**:277-290.

16. Ren G, Healy RA, Horner HT, Martha GJ, Thornburg RW: **Expression of starch metabolic genes in the developing nectaries of ornamental tobacco plants**. *Plant Sci* 2007, **173**:621-637.

17. Lüttge U: **Über die Zusammensetzung des Nektars und den Mechanismus seiner Sekretion. I**. *Planta* 1961, **56**:189-212.

18. •• Wenzler M, Holscher D, Oerther T, Schneider B: **Nectar formation and floral nectary anatomy of *Anigozanthos flavidus*: a combined magnetic resonance imaging and spectroscopy study**. *J Exp Bot* 2008, **59**:3425-3434.

NMR techniques were used to investigate the composition of nectar sugars. A deviation from the expected 1:1 ratio of the hexoses glucose and fructose can be explained by prior cycling of sucrose through complex biochemical pathways before secreting into floral nectar.

19. Herrera CM, Garcia IM, Perez R: **Invisible floral larcenies: microbial communities degrade floral nectar of bumble bee-pollinated plants**. *Ecology* 2008, **89**:2369-2376.

20. Carter C, Thornburg RW: **Is the nectar redox cycle a floral defense against microbial attack?** *Trends Plant Sci* 2004, **9**:320-324.

21. Raguso RA: **Start making scents: the challenge of integrating chemistry into pollination ecology**. *Entomol Exp Appl* 2008, **128**:196-207.

22. Tanaka Y, Sasaki N, Ohmiya A: **Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids**. *Plant J* 2008, **54**:733-749.

23. Goyret J, Markwell PM, Raguso RA: **Context- and scale-dependent effects of floral CO_2 on nectar foraging by *Manduca sexta***. *Proc Natl Acad Sci U S A* 2008, **105**:4565-4570.

24. Chalcoff VR, Aizen MA, Galett L: **Sugar preferences of the green-backed firecrown hummingbird (*Sephanoides sephaniodes*): a field experiment**. *Auk* 2008, **125**:60-66.

25. Lotz CN, Schondube JE: **Sugar preferences in nectar- and fruit-eating birds: behavioral patterns and physiological causes**. *Biotropica* 2006, **38**:3-15.

26. Kromer T, Kessler M, Lohaus G, Schmidt-Lebuhn AN: **Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae**. *Plant Biol* 2008, **10**:502-511.

27. Wolff D: **Nectar sugar composition and volumes of 47 species of *gentianales* from a southern Ecuadorian montane forest**. *Ann Bot* 2006, **97**:767-777.

28. Johnson SD, Nicolson SW: **Evolutionary associations between nectar properties and specificity in bird pollination systems**. *Biol Lett* 2008, **4**:49-52.

29. Fleming PA, Bakken BH, Lotz CN, Nicolson SW: **Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird**. *Funct Ecol* 2004, **18**:223-232.

30. Fleming PA, Xie S, Napier K, McWhorter TJ, Nicolson SW: **Nectar concentration affects sugar preferences in two Australian honeyeaters and a lorikeet**. *Funct Ecol* 2008, **22**:599-605.

31. Schiestl FP: **On the success of a swindle: pollination by deception in orchids**. *Naturwissenschaften* 2005, **92**:255-264.

32. Jersakova J, Johnson SD, Kindlmann P: **Mechanisms and evolution of deceptive pollination in orchids**. *Biol Rev* 2006, **81**:219-235.

33. Schluter PM, Schiestl FP: **Molecular mechanisms of floral mimicry in orchids**. *Trends Plant Sci* 2008, **13**:228-235.

34. •• Vereecken NJ, Schiestl FP: **The evolution of imperfect floral mimicry**. *Proc Natl Acad Sci U S A* 2008, **105**:7484-7488.

Orchids fool male bees by emitting the female bees sex pheromones. Both female bees and orchids produce the same compounds to attract mating partners, but in different relative proportions. Male bees significantly prefer orchids to females, probably due to their sensory preference of 'novel' signals.

35. •• Peter CI, Johnson SD: **Mimics and magnets: the importance of color and ecological facilitation in floral deception**. *Ecology* 2008, **89**:1583-1595.

The close resemblance to the model species often explains the success of mimicking food deceptive orchids. Using a bee vision model, the spectral reflectance of the petals was analyzed. The results indicate that bees are not able to distinguish between mimic and model.

36. Jersakova J, Johnson SD: **Lack of floral nectar reduces self-pollination in a fly-pollinated orchid**. *Oecologia* 2006, **147**:60-68.

37. Jersakova J: **Do nectar manipulation studies support the cross-pollination hypothesis?** *S Afr J Bot* 2008, **74**:369-1369.

38. Anderson B, Johnson SD: **The effects of floral mimics and models on each others' fitness**. *Proc Roy Soc B-Biol Sci* 2006, **273**:969-974.

39. Anderson B, Johnson SD: **The geographical mosaic of coevolution in a plant–pollinator mutualism**. *Evolution* 2008, **62**:220-225.

40. •• Jersakova J, Johnson SD, Kindlmann P, Pupin AC: **Effect of nectar supplementation on male and female components of pollination success in the deceptive orchid *Dactylorhiza sambucina***. *Acta Oecol-Int J Ecol* 2008, **33**:300-306.

Nectar supplementation had an overall positive effect on reproductive success of deceptive orchids, regardless of petal color. It is assumed that a mutation resulting in nectar production (as observed in other orchid species) might not have occurred because enhanced nectar volumes can cause inbreeding and involve costs.

41. Leiss KA, Vrieling K, Klinkhamer PGL: **Heritability of nectar production in *Echium vulgare***. *Heredity* 2004, **92**:446-451.

42. Herrera CM, Perez R, Alonso C: **Extreme intraplant variation in nectar sugar composition in an insect-pollinated perennial herb**. *Am J Bot* 2006, **93**:575-581.

43. Goulson D, Cruise JL, Sparrow KR, Harris AJ, Park KJ, Tinsley MC, Gilburn AS: **Choosing rewarding flowers; perceptual limitations and innate preferences influence decision making in bumblebees and honeybees**. *Behav Ecol Sociobiol* 2007, **61**:1523-1529.

44. Canto A, Perez R, Medrano M, Castellanos MC, Herrera CM: **Intra-plant variation in nectar sugar composition in two *Aquilegia* species (Ranunculaceae): contrasting patterns under field and glasshouse conditions**. *Ann Bot* 2007, **99**:653-660.

45. Carlson JE: **Hummingbird responses to gender-biased nectar production: are nectar biases maintained by natural or sexual selection?** *Proc Roy Soc B-Biol Sci* 2008, **275**:1717-1726.

46. Smith SD, Ane C, Baum DA: **The role of pollinator shifts in the floral diversification of iochroma (Solanaceae)**. *Evolution* 2008, **62**:793-806.

47. Martins DJ, Johnson SD: **Hawkmoth pollination of aerangoid orchids in Kenya, with special reference to nectar sugar concentration gradients in the floral spurs**. *Am J Bot* 2007, **94**:650-659.

48. Stuurman J, Hoballah ME, Broger L, Moore J, Basten C, Kuhlemeier C: **Dissection of floral pollination syndromes in *Petunia***. *Genetics* 2004, **168**:1585-1599.

49. •• Galliot C, Hoballah ME, Kuhlemeier C, Stuurman J: **Genetics of flower size and nectar volume in *Petunia* pollination syndromes**. *Planta* 2006, **225**:203-212.

The genetic architecture of differences in floral size and nectar volume are studied in two closely related *Petunia* species. An AFLP-based QTL map was established to define the genomic regions explaining for phenotypical variation. QTLs with moderate and small effects underlying nectar and size suggest the polygenic nature of these floral traits.

50. Bradshaw HD, Wilbert SM, Otto KG, Schemske DW: **Genetic-mapping of floral traits associated with reproductive isolation in monkeyflowers (*Mimulus*)**. *Nature* 1995, **376**:762-765.