Topic 4
REWARDS IN FLOWERS

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MAEC

Biology of plant–insect interactions

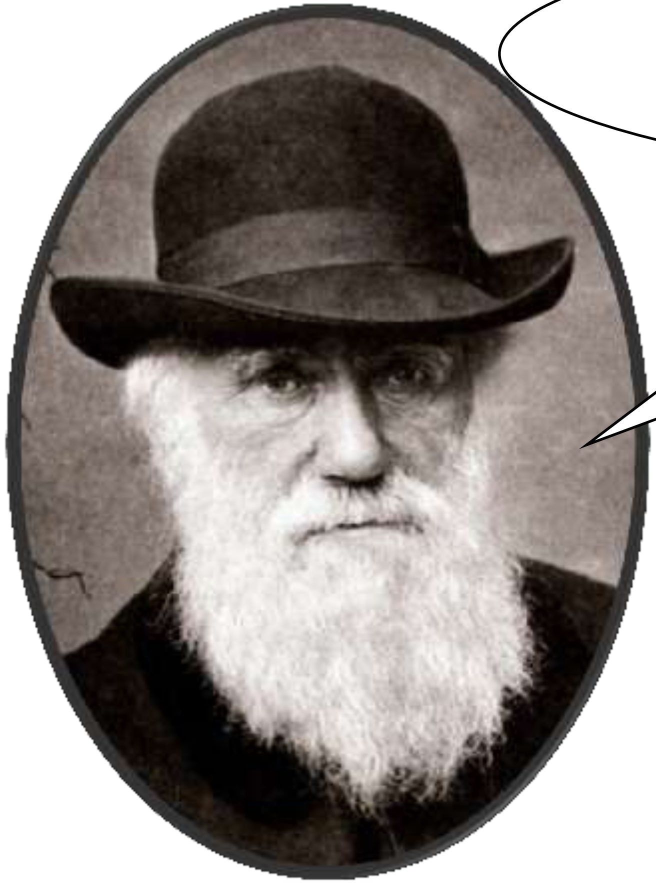
CONTENT OF TOPIC 4 «Rewards in flowers»

1. Consideration about the terminology
2. Contrasting needs of plants and animals
3. Types of rewards
4. Costs, benefits and risks in producing a reward
5. Deceit in pollination
6. Pollen as reward

7. Nectar as reward
8. Nectar traits: volume & concentration
9. Nectar chemical composition: Sugars, amino acids, SMs
10. The «manipualtion» theory

11. Practical methods for studying nectar in the field
12. How to measure volume and concentration
13. Different methods for nectar storing
14. Nectar Energetics

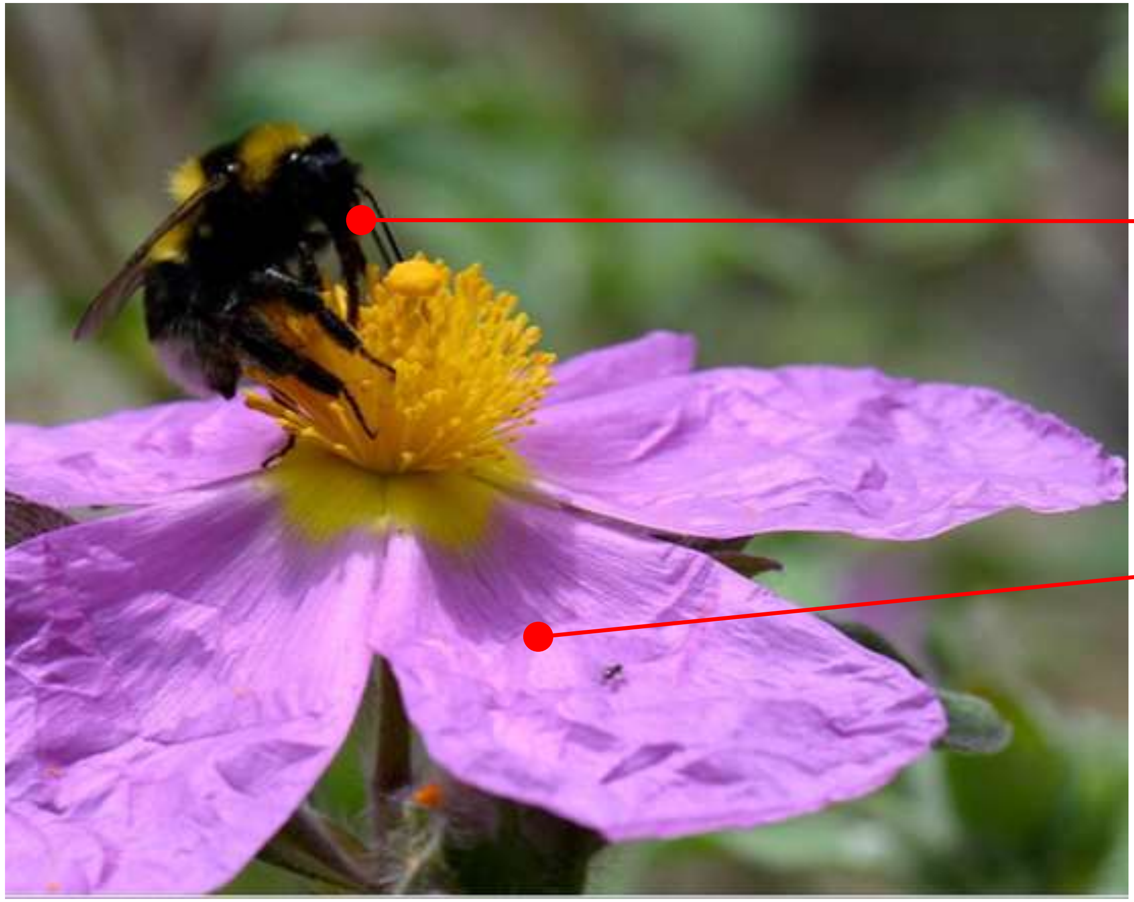
Reward?



“natural selection cannot possibly produce any modification in a species exclusively for the good of another species.” (Darwin, 1859)

There is no any kind of altruism in plant-pollinator interactions

ATTRACTANT (Dafni A. 1995) could be a better terminology mutualistic relationships hide an apparent paradox since each species tends to maximize the benefits and its own fitness when interacting with another and unrelated partners may have conflicts of interests



Insects maximize the intake of food reducing the expenditure for foraging. Pollination is unintentional

Flowers (plants) maximize the probability of pollination with the less investment in providing reward for efficient pollination attraction

Strong conflict of interest between flowers and pollinators

Mutualism → Exploitation

Why animals visit flowers?

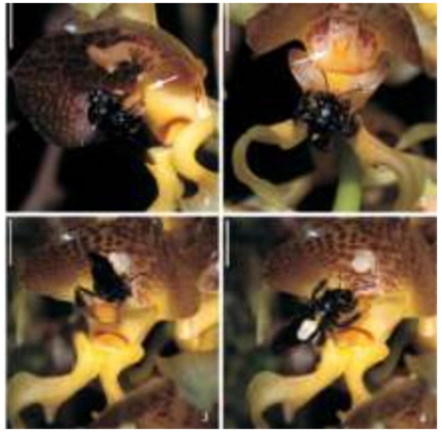
- primary attractans

For a miriad of reasons, but not to pollinate

Table 1. Types of reward in flowers and their implications in pollination

The reward	Chemical composition	Users	Ecological implications
Nectar	Carbohydrates, amino acids, lipids, antioxidants, alkaloids, protein, vitamins, and others	Almost all the known pollinator groups, especially for the forager's own consumption	The main calorific reward. Nectar volume, concentration, rate and rhythms of secretion are ± typical to the pollinator group. There is a correlation between the floral morphology (tube) and the presence/quantity/quality of the nectar
Pollen	Proteins, carbohydrates, amino acids, lipids, minerals, enzymes, pigments, and others	Especially insects, also used as a main food of the brood	In large grains starch is the energetic source but it is lipids in small ones. Pollen is a convenient food source which requires a minimum of adaptations on the part of the users; almost every mandibulate insect may use it
Stigmatic exudates	Lipids, sugars, amino acids, phenolics, alkaloids, and anti-oxidants	Insects	The main reward in only a few cases, especially in trap flowers
Floral tissues	Sugar, starch, protein, lipids, etc.	Insects (mainly beetles and bees). Bats	Special food-bodies, false anthers, or other unspecialized tissues (petals, etc.)
Oils	Saturated free fatty acids, diglycerides	Specialized female bees (Anthophorinae; Old Tropics, Rediviva; Mellittidae) South Africa	Only in specialized flowers mainly in the tropics. Produced in special structures (elaiophores). Oil collection requires the use of specialized structures formed by modified setae and fore tarsi

From Dafni A. 1995. Pollination Ecology – A practical approch



Hymenoptera Lepidoptera Diptera Mammals (bats) birds

Why animals visit flowers?

• secondary attractans

Perfume	Terpenes (e.g. cineole, geraniol, eugenol) Aminoid compounds (e.g. skatole), aromatic constituents (e.g. vanillin)	Male euglossine bees	Produced especially by Orchidaceae but also Araceae. The male stores the liquid in a special tissue in his hind tibia, but its exact metabolism and function is not known for sure
Resins and gums	Terpenes	Bees (Euglossini, Meliponini; Anthidiini); only females	Produced by a few plant species in the tropics. The bees use the material for nest construction
Brood sites		Highly specialized insects	Oviposition site and brood rearing in the flower (*Yucca*, *Ficus*); the adults are the most important or the only pollinators of the flowers involved
Shelter and heating	The flower offers shelter and/or heating	Insects (bees, flies, beetles)	Insects which sleep or stay at the flowers may get an energetic gain since the flower is warmer than the ambient temperature
	Parabolic flowers act as diaheliotropic solar furnaces	Insects	Insects bask in the flowers, which are warmer than the surrounding air, and make an energy gain
Meeting-places (rendezvous)	Higher chance for mates	Insects	Males which are seeking mates in the flower also pollinate them
Prey	The flower attracts prey	Insects	The predator serves also as a pollinator?

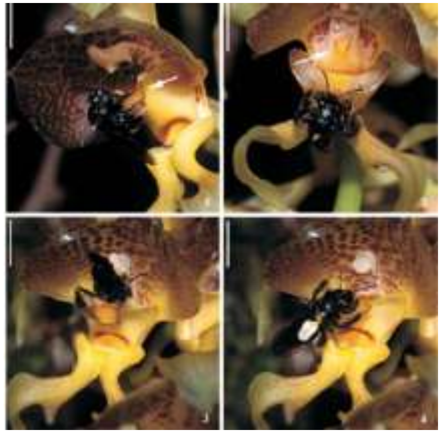
Sources: refs 1–10.

From Dafni A. 1995. Pollination Ecology – A practical approch

COST AND BENEFITS FOR PRODUCING A REWARD

Pollen is the cheapest reward, since it must be produced by the plant regardless the interaction with pollinators. Pollen was thought to be the reward for pollinators in the flowers of very early first angiosperms.

Stigmatic exudate is the reward for pollinators in early diverging angiosperme lineages. At the same time stigmatic exudates are responsible for promoting pollen germination.



All the other primary attractant (nectar, oils, floral tissues) are not essential for the plant, they are a surplus production and this means a cost.

Nectar is very costly, since its high sugar content. Up to 37% of the daily photosynthate can be invested in nectar production

This (high) extracost is compensated by the increased attractiveness of flowers, and thus possibly by an increased pollination service.

plants have to balance the trade-off between nectar production and plant growt-reproduction

THE RISKS OF PRODUCING A REWARD

Alluring rewards for mutualists may also attract exploiters, i.e. organism that consume nectar without performing pollination

Plants face a dilemma: How to attract mustualist (pollinators) and repel exploiters?

They solved the dilemma by adjusting the chemistry of nectar

Making sense of nectar scents: the effects of nectar secondary metabolites on floral visitors of *Nicotiana attenuata*

Danny Kessler, Ian T. Baldwin

the plant journal 2006, 49: 840-854

First published: 20 February 2007 | https://doi.org/10.1111/j.1365-313X.2006.02995.x

in modulating visitors' behavior. Kessler et al. [33] demonstrated that the repellent nicotine and the attractant benzyl acetone are both necessary for maximizing fruit production and flower visits by native pollinators, while nicotine reduces theft of nectar by non-pollinating animals. Nicotine is a typical insect-repelling alkaloid that maximizes the number of flower visitors per unit volume of floral nectar secreted by *Nicotiana attenuata*. In this way plants can minimize nectar volumes, whilst maximizing pollination efficiency and seed production [34]. Singaravelan et al. [35] demon-

THE RISKS OF PRODUCING A REWARD

Evolutionary Ecology Research, 2002, 4: 293–305

Do extrafloral nectaries distract ants from visiting flowers? An experimental test of an overlooked hypothesis

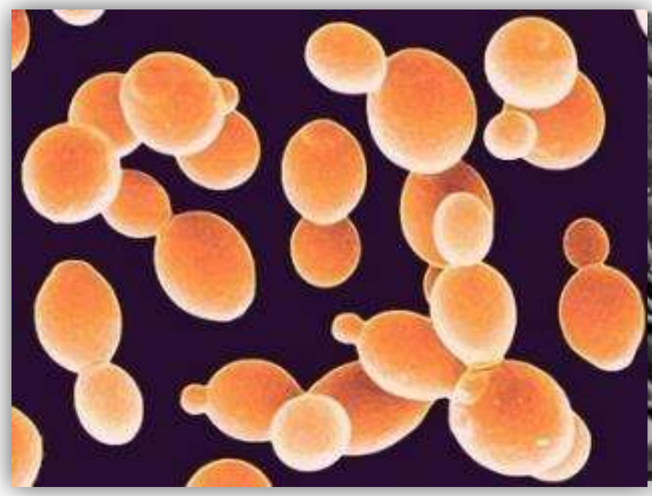
Diane Wagner[1]* and Adam Kay[2‡]

Ants are considered exploiters of floral nectar (nectar thieves), since they were frequently observed collecting nectar without performing pollination.

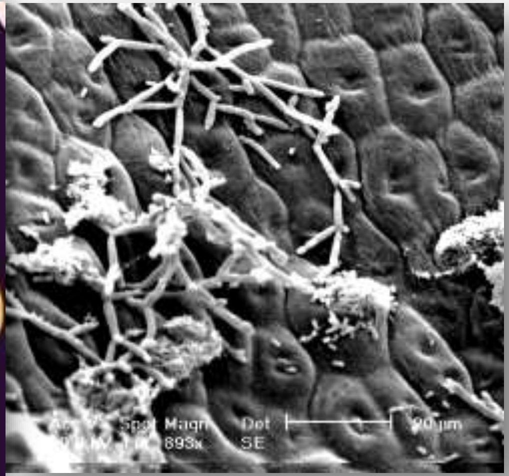
Extrafloral nectaries in angiosperms may had evolved in order to distract ants from visiting the flowers

THE RISKS OF PRODUCING A REWARD

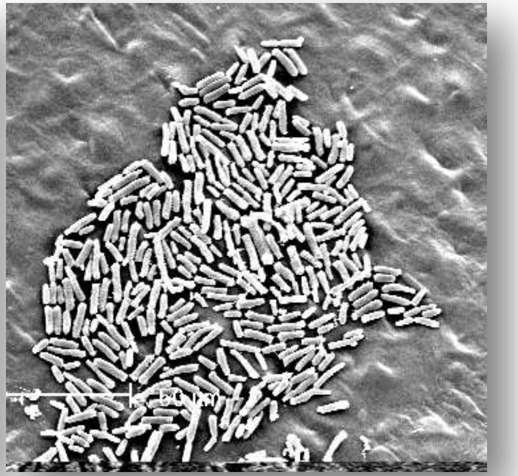
Nectar can be the medium for microorganisms' growth..... For Metschnikowiaceae yeasts nectar is the elective habitat



Yeasts



Filamentous fungi



bacteria

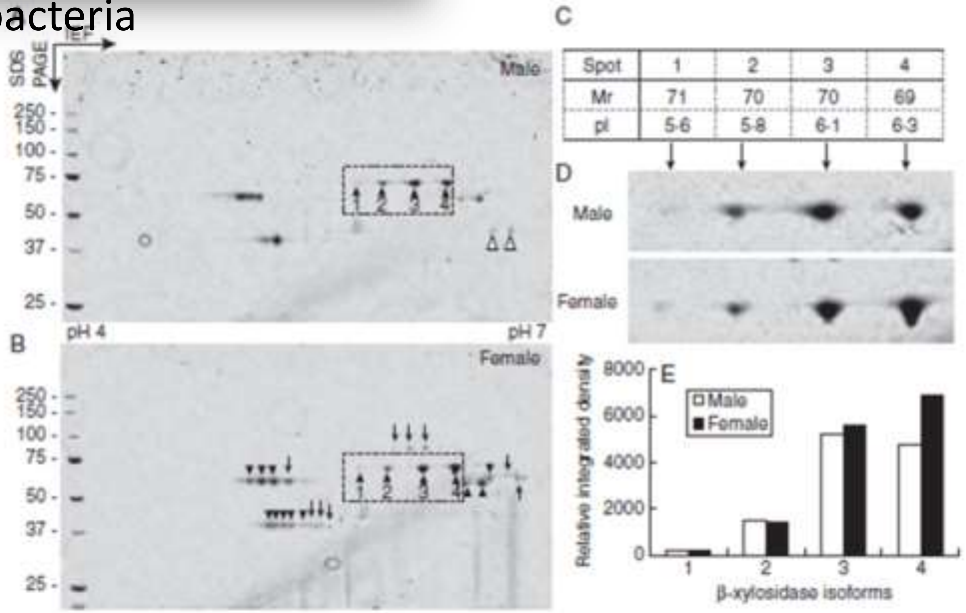
Sugar and amino acid consumption

fermentation

Interaction with animals

Ethanol, volatile substances

Nectar proteins with antimicrobial and antifungal activity



POLLINATION BY FOOD DECEIT

Prosoeca ganglbaueri pollinate nectar producing *Zaluzianskya microsiphon (Scrophulariaceae)* and rewardless mimic *Disa nivea*

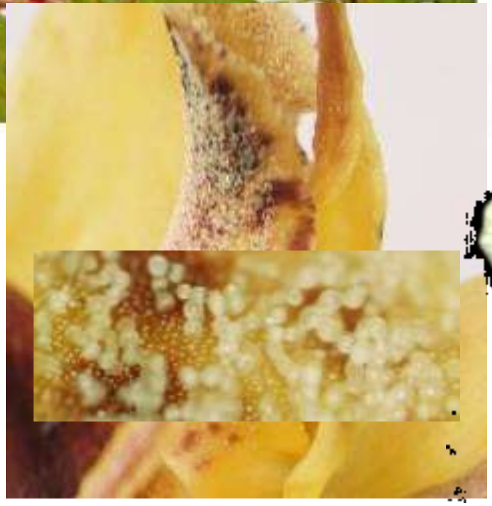
Batesian mimicry

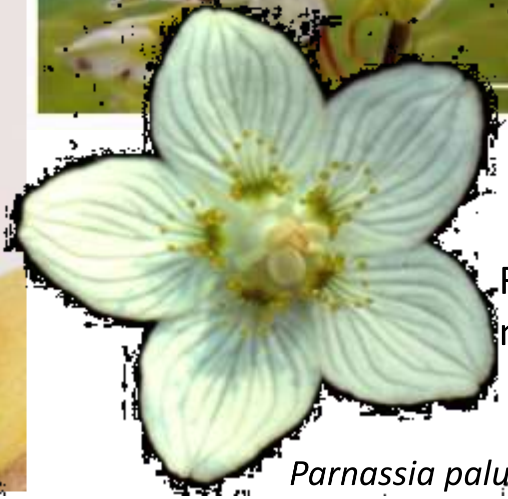
An unrewarding species mimic a rewarding species (model plant)

- Model and mimetic plant are sympatric
- Frequency of mimetic plant is lower
- Reproduction success of mimetic plant is higher in the presence of model
- Similar floral signals, difficult to recognize for common pollinator

Deceptive food


deceptive resin


Pseudo-pollen


False nectar

Parnassia palustris

WHEN INSECTS DECEIVE THE PLANTS…..

Nectar Robbing behaviour

Xylocopa

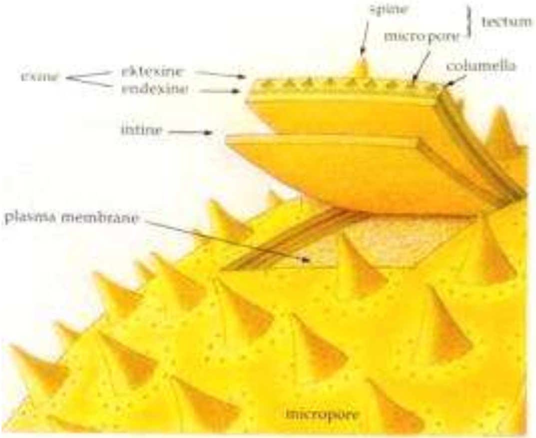
Linaria vulgaris

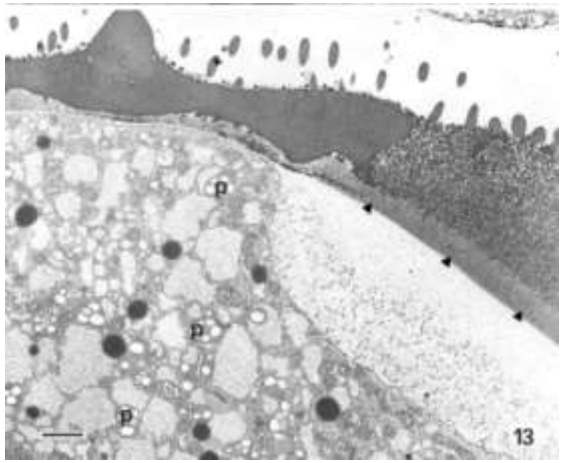
Bombus

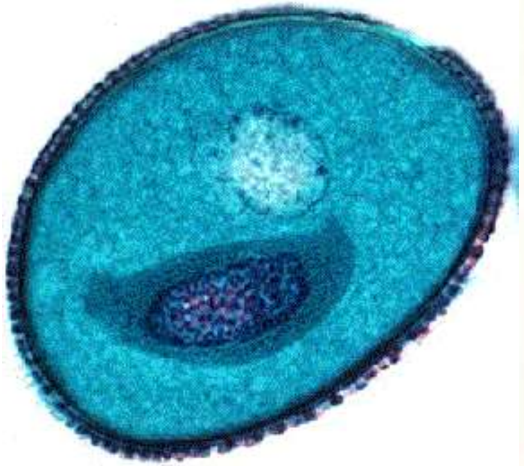
Primary and secondary nectar robbing

POLLEN AS REWARD

the male gametophyte is a dispersal structure: as such it is rich in nutrients (protein, amino acids, carbohydrates, lipids) but they are hiddden and protected







sporoderm

:

EXINE: sporopollenin

INTINE cellulose & pectins

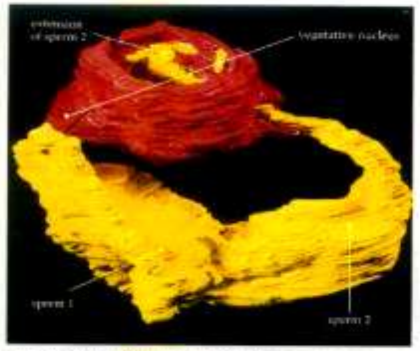
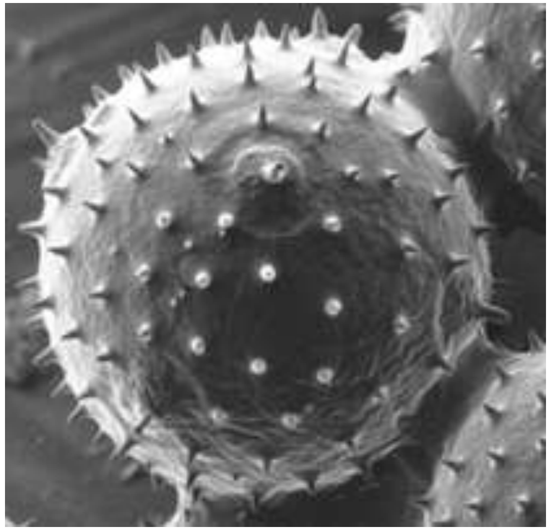
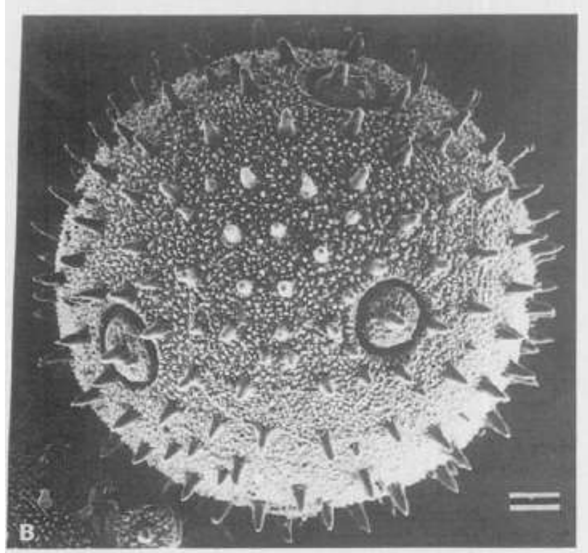


Fig. 15.5 The male germ unit provides a means for sperm transport in the pollen tube. Three-dimensional reconstruction of a male germ unit in a pollen grain of oilseed rape, *Brassica campestris*. The pair of sperm cells are linked together and one has a long extension that penetrates the vegetative nucleus in mature pollen



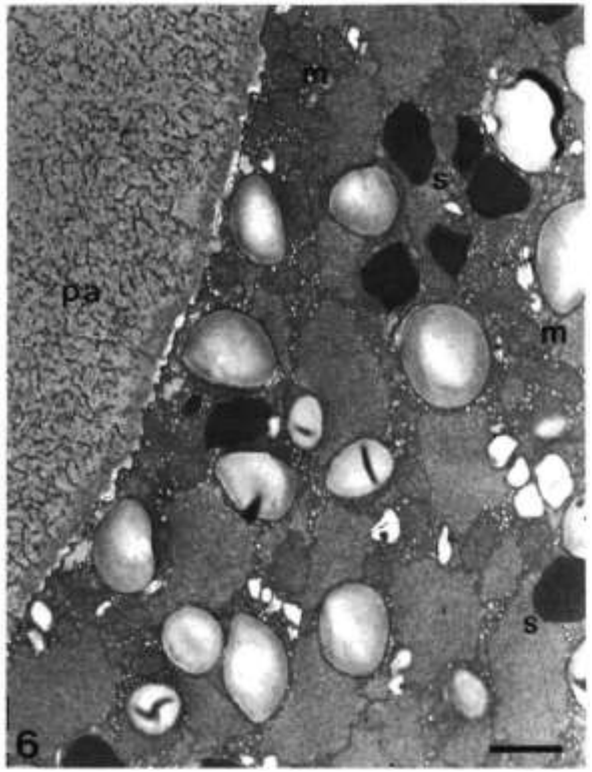
POLLENKITT

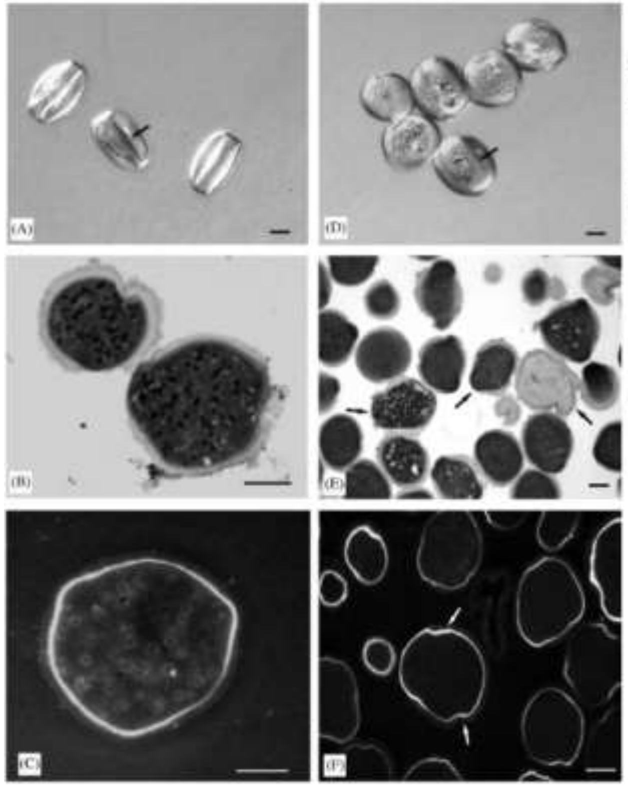


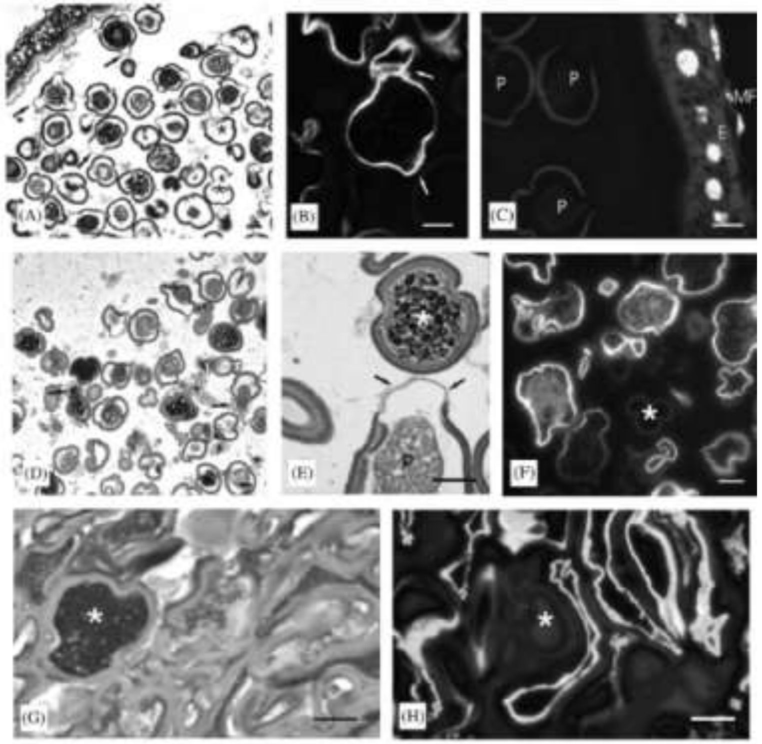
HOW TO EXTRACT NUTRIENTS FROM POLLEN GRAINS?

Thrips can pierce the pollen grains and directly access the nutrients.

Bees are usual to use osmotic shock to access the pollen cytoplasm







From the anther to the proctodeum: Pear (*Pyrus communis*) pollen digestion in *Osmia cornuta* larvae

Massimo Nepi[a], Laura Cresti[a], Bettina Maccagnani[b,*], Edith Ladurner[b], Ettore Pacini[a]

Journal of Insect Physiology 51 (2005) 749–757

POLLEN COLLECTION

on by *Megachile zapoteca*. **a** *Megachile zapoteca* landing on the petals'
n. **c** Introducing its proboscis in the insertion point between the
plex. **d**, **e** Staminal column and gynoecium are explosively disclosed,
the bee (arrow). **f** Bee leaves after tripping the flower. Scale bar 2 mm

ding system of *Desmodium*
noideae): functional aspects

INSECT SPECIALIZATION IN POLLEN COLLECTION

POLYLECTIC INSECTS

Insects that forage on a variety of pollen resources (althgough with a certain floral constancy at individual level)

OLIGOLECTIC-MONOLECTIC INSECTS

Insects that forage on few or only one plant species (or from one plant genus)

Colletes anchusae

Cynoglottis barellierii

HOW PLANTS ATTRACT THE VERY SPECIALIZED OLIGOLECTIC BEES?

It is maily a matter of a «private channel» communication by uncommon scents that are not perceived by other insects.

~ 20,000 species of angiosperms are strictly pollen-rewarding

There is a paradox: Every grain of pollen consumed by a pollinator is a lost reproductive opportunity

Nectar addition changes pollinator behavior but not plant reproduction in pollen-rewarding *Lupinus argenteus*

Jacob M. Heiling[1,2,3,4,6], Judith L. Bronstein[2,5], and Rebecca E. Irwin[1,2]

RESULTS: Bees exhibited behavioral responses to the novel reward, collecting nectar as well as pollen and spending 27% longer per flower. Pollen transfer increased with flower visit duration. However, plants in the study population were not pollen-limited; consequently, the observed changes in pollinator behavior did not result in changes in female components of plant reproduction.

American Journal of Botany 2021, 108: 1-9

Bees remember flowers for more than one reason: pollen mediates associative learning

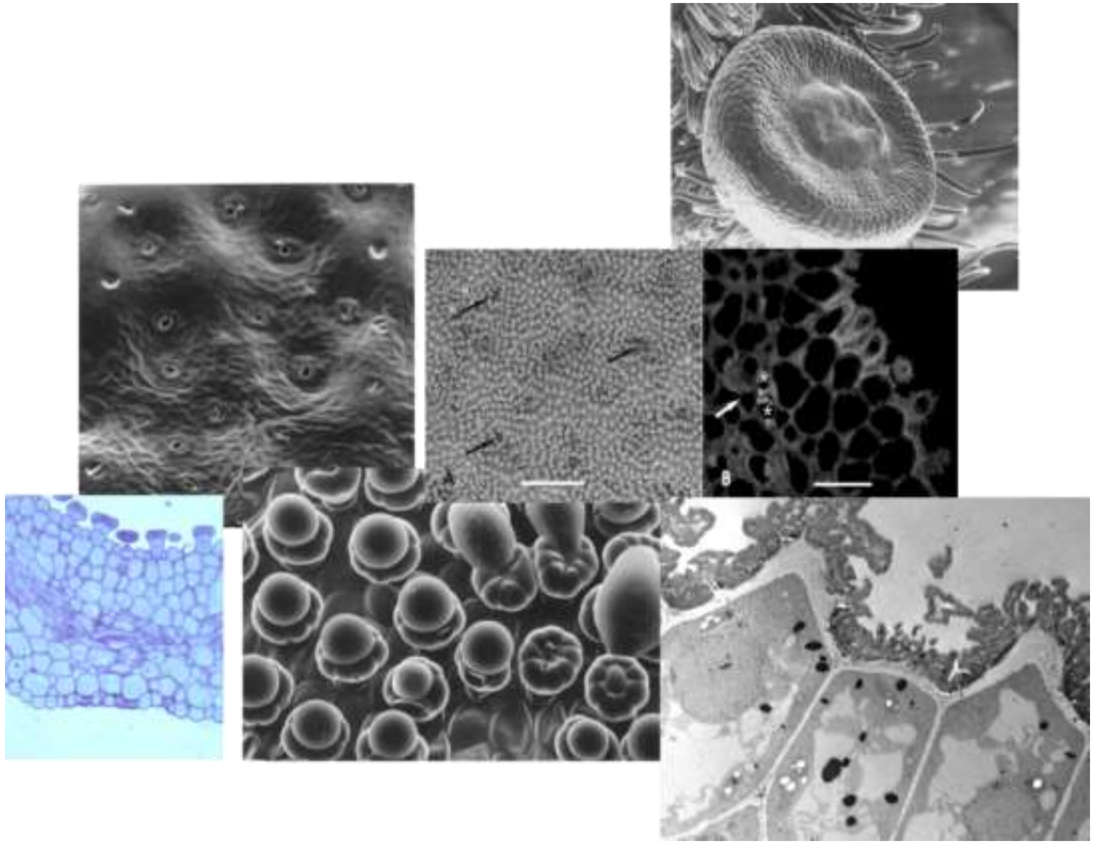
Felicity Muth[a,*], Daniel R. Papaj[b], Anne S. Leonard[a] Animal Behaviour 111 (2016) 93–100

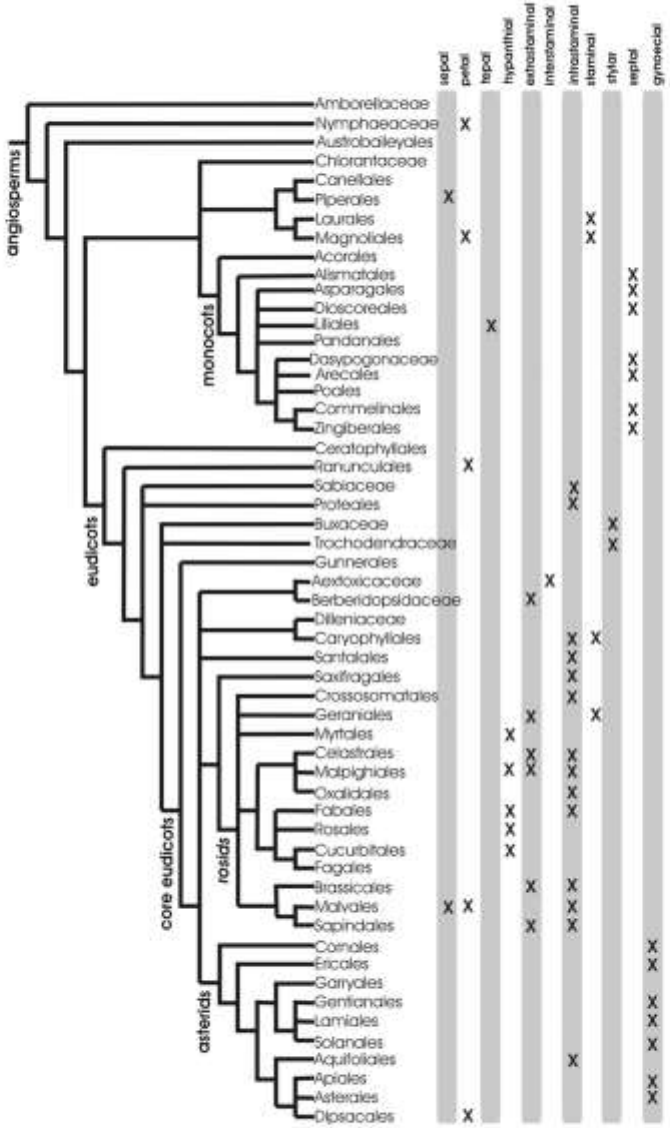
rewards is unknown. Here we show that bees can learn to associate multiple floral features with a pure pollen reward. Furthermore, these associations are remembered long term, comparable to bees' memory for nectar associations. These findings raise new questions about bee learning and the evolutionary history between plants and bee pollinators.

....Moreover, pollen rewards are taxonomically widespread (Vogel, 1978) and pre-date the evolution of nectar (Crepet, Friis, Nixon,

NECTAR AS REWARD

Nectar is sugary secretion produced by special organs, called nectaries, that can be highly diverse concerning topography, anatomy and structure. Nectaries are very common in Angiosperms

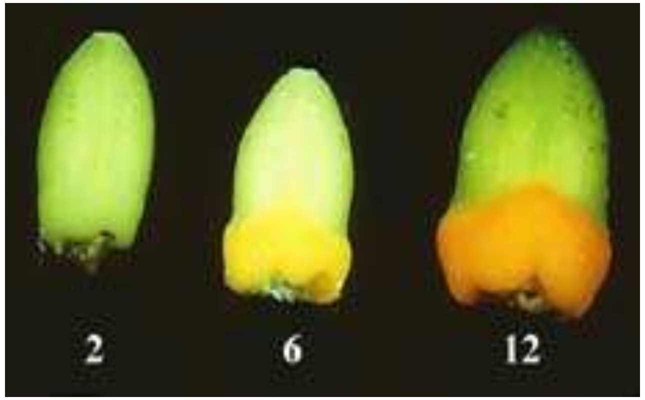




Beranardello G. 2007. A systematic survey of floral nectaries. In: Nectaries and nectar. Nicolson S.W., Nepi M., Pacini E. (eds.), Springer: Dordrecht. pp.19-128

HOW TO LOCATE NECTAR?

Friendly and cohoperative flowers



unfriendly and uncohoperative flowers

+ Ruthenium red staining

NECTAR TRAITS: VOLUME, CONCENTRATION, TOTAL SUGARS

VOLUME

The volume of nectar is generally relates to the size of the pollinators: larger animals generally require higher nectar volume. The highest volume are registered in flowers pollinated by birds and bats.

CONCENTRATION

Bird pollinated flowers commonly produce large quantity of diluted nectar, whereas insect pollinated flowers produce low volume of high concentrated nectar since their very high metabolic rate.

TOTAL SUGARS per flower

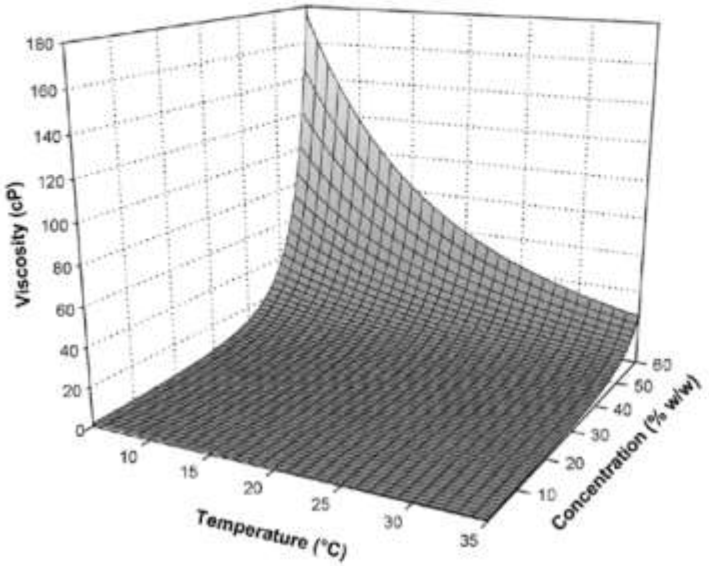
After converting the concentration from % w/w to mg/µl (convertion tables) it is possible to calculate the total sugar quantity per flower:
nectar volume (µl) * concentration (mg/µl) = mg of sugar per flower
It represents the rel investment of flower in the production of nectar

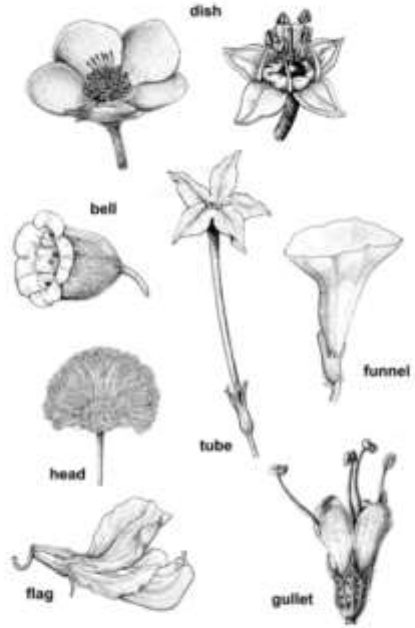
NECTAR TRAITS: VOLUME, CONCENTRATION, TOTAL SUGARS

Nectar volume and concentration are highly affected by T and RH of the air around the flower

High T and low RH values decrease nectar volume and increase concentration because water loss by evaporation.
POST SECRETORY CHANGES OF NECTAR

EFFECT ON NECTAR VISCOSITY ⇒ EFFECT ON FEEDING RATE AND FORAGING EFFICIENCY





The effect of t & RH can be reduced by:

- morphological features of flowers that offer more protection to the nectar (i.e. long corolla tubes, spurs
- waterproofing lipid monolayers;
- reabsorption of sugar
- constant high secretion rate of diluted nectar

NECTAR TRAITS: VOLUME, CONCENTRATION, TOTAL SUGARS

Interspecific and intraspecific variability of nectar traits

There is a wide range of interspecific variability in nectar volume and concetration. This variability have been interpreted as adaptive: pollinators behaviour favours certain traits over other.

A broad intraspecific variability was also demonstrated at different level:

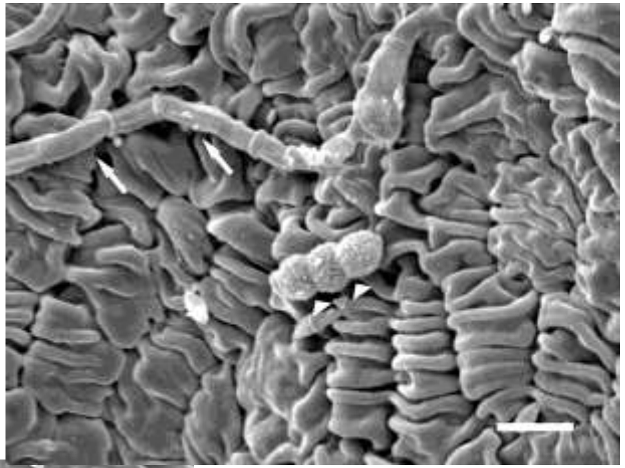
- Between nectaries within individual flowers
- Between flowers within Individual plants (position in the inflorescence, micro-environ. around flowers, flower age, sexual phase). A variable fraction of flowers within individuals are void of nectar.
- Between plants within a population (genetic diversity, dioecious species, dimorphic species)
- Between populations

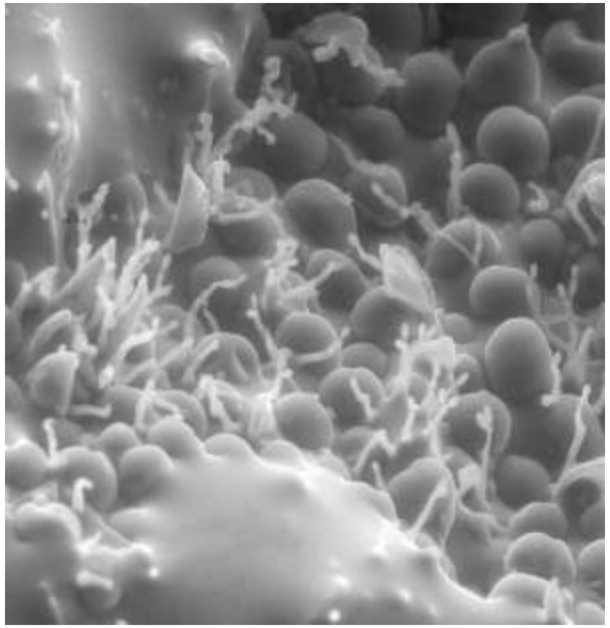
Many researchers argue that nectar variability may itself be adaptive because pollinators encountering variable rewards are more likely to move from plant to plant, promoting outcrossing

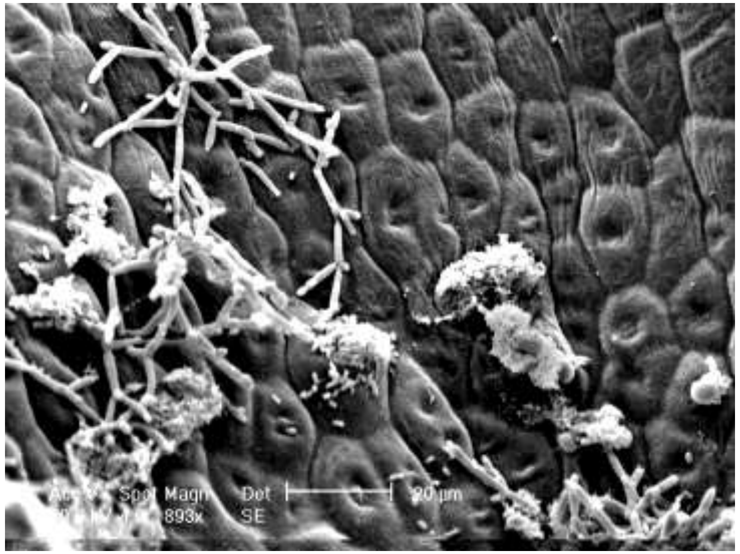
NECTAR TRAITS: VOLUME, CONCENTRATION, TOTAL SUGARS

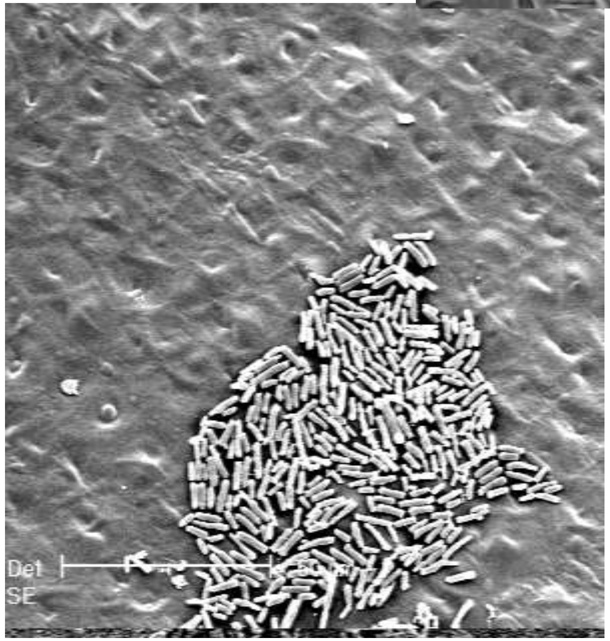
Pollinators may contaminate nectar by transporting fungi or bacteria, or the airborne spores of these organisms may directly contaminate nectar.

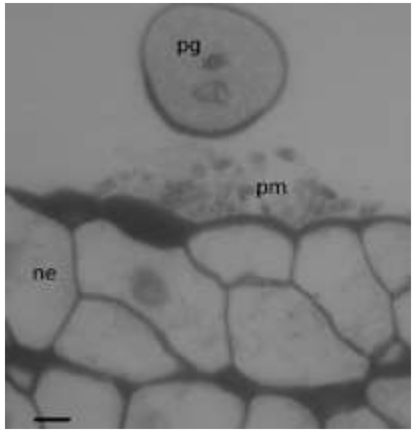
These microorganisms exploit nectar nutrients and are responsible for the decrease in nectar concentration (as well as a modification of the sugar and amino acid profile)







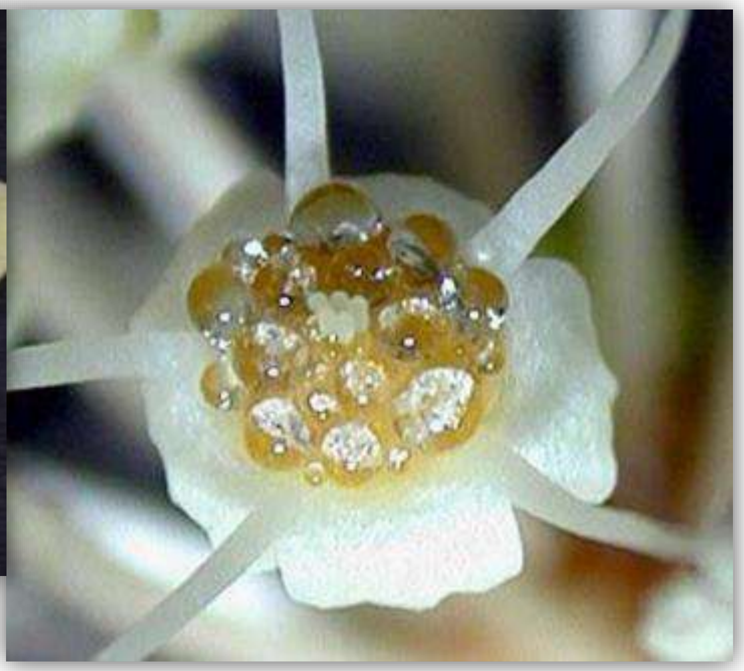




Why a so large variety of animals collect and feed on nectar?

Being simple sugars the main components, it is a very energetic drink that can be easily collected and absorbed (it does not require digestion)





Glucose Fructose Sucrose

THE BIG THREE

convergence of nectar chemestry driven by pollinators

Table 5-10. Numbers of species in each of the four sugar-ratio categories arranged by predominant pollinators.

	$\frac{S}{G+F}$						
	< 0.1	0.1 to 0.499	0.5 to 0.999	> 0.999	*N*	*G**	*P***
OVERALL	195	231	149	190	765	—	—
Hummingbirds	0	18	45	77	140	119.52	< .001
New World passerines	11	1	0	0	12	25.16	< .001
Sunbirds, etc.	24	9	2	0	35	28.07	< .001
Honeyeaters	18	4	0	0	22	36.87	< .001
Honeycreepers	5	1	0	0	6	10.57	< .02
Lorikeets, etc.	1	2	0	0	3	3.69	.30
Hawkmoths	2	8	19	32	61	41.16	< .001
Settling moths	3	14	11	15	43	70.07	< .001
Butterflies and skippers	5	17	24	29	75	24.23	< .001
Short-tongued bees and butterflies	23	21	3	0	47	38.07	< .001
Short-tongued bees	115	103	28	17	263	75.47	< .001
Long-tongued bees	13	75	49	66	203	42.40	< .001
New World bats	9	18	0	0	27	32.51	< .001
Old World bats	1	3	2	1	7	1.36	.90
Nonvolant mammals	0	2	2	1	5	13.44	< .01
Wasps	2	7	4	5	18	1.24	.75
Beetles	1	3	2	3	9	1.22	.75
Flies	29	27	7	9	72	14.82	< .001

*G = G-statistic (see text).
**P = probability of difference from OVERALL.

Baker HG and Baker I. 1983. Floral nectar sugar constituents in relation to pollinator type. In: “Handbook of pollination Biology” Jones CE and Littles RJ.

Scientific and Academic Edition, New York.

NECTAR TRAITS: COMPOSITION

Amino acids, after sugars, are the more abundant components of nectar.
They contribute to the taste of nectar

the effects of amino acids on insect chemoreceptors has permitted the description of four taste classes of amino acids:

Class I (Asn, Gln, Ala, Cys, Gly, Ser Thr, Tyr) have no effect on the chemoreceptors.
Class II (Arg, Asp, Glu, His, Lys) are generally inhibitory to insect chemoreceptors.
Class III (Pro and Hyp) have the unique ability to stimulate the salt cell (increased feeding behavior).
Class IV (Ile, Leu, Met, Phe, Trp, Val) includes amino acids with the ability to stimulate the sugar cell (increased attraction to sugar rich solution)

Proline in honeybee:

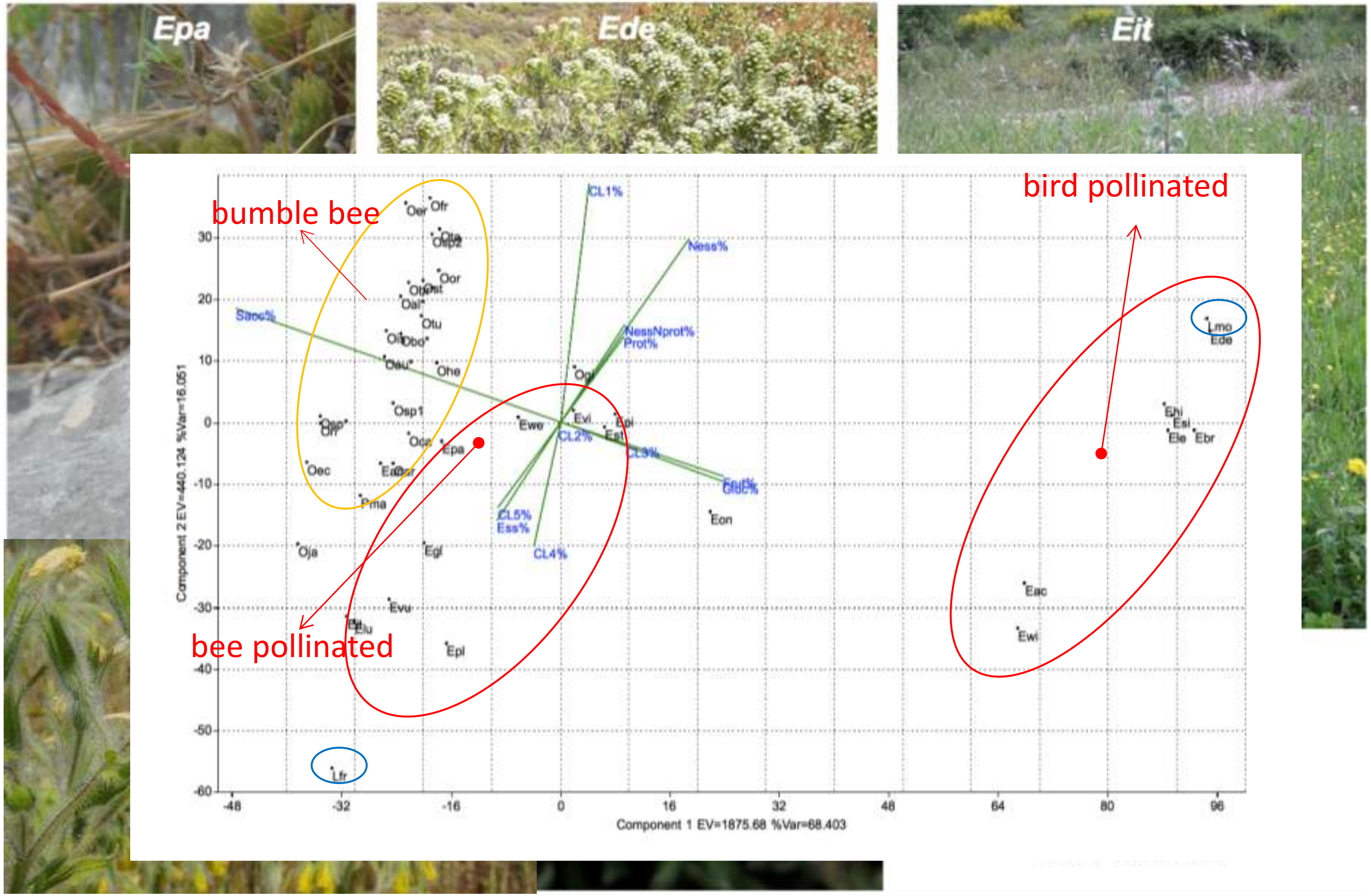
- most abundant amino acid in the hemolymph
- necessary for egg laying
- provides a rapid, short burst of energy

20 Protein amino acids

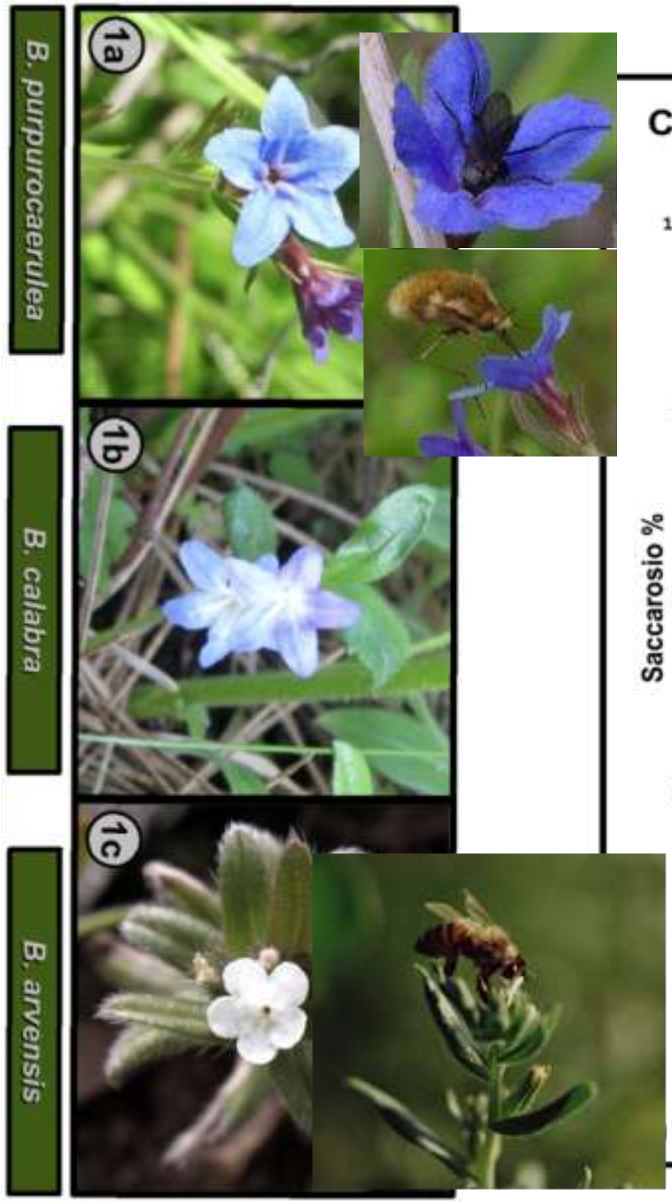
Hundreds non-protein Aminoacids (secondary compounds)

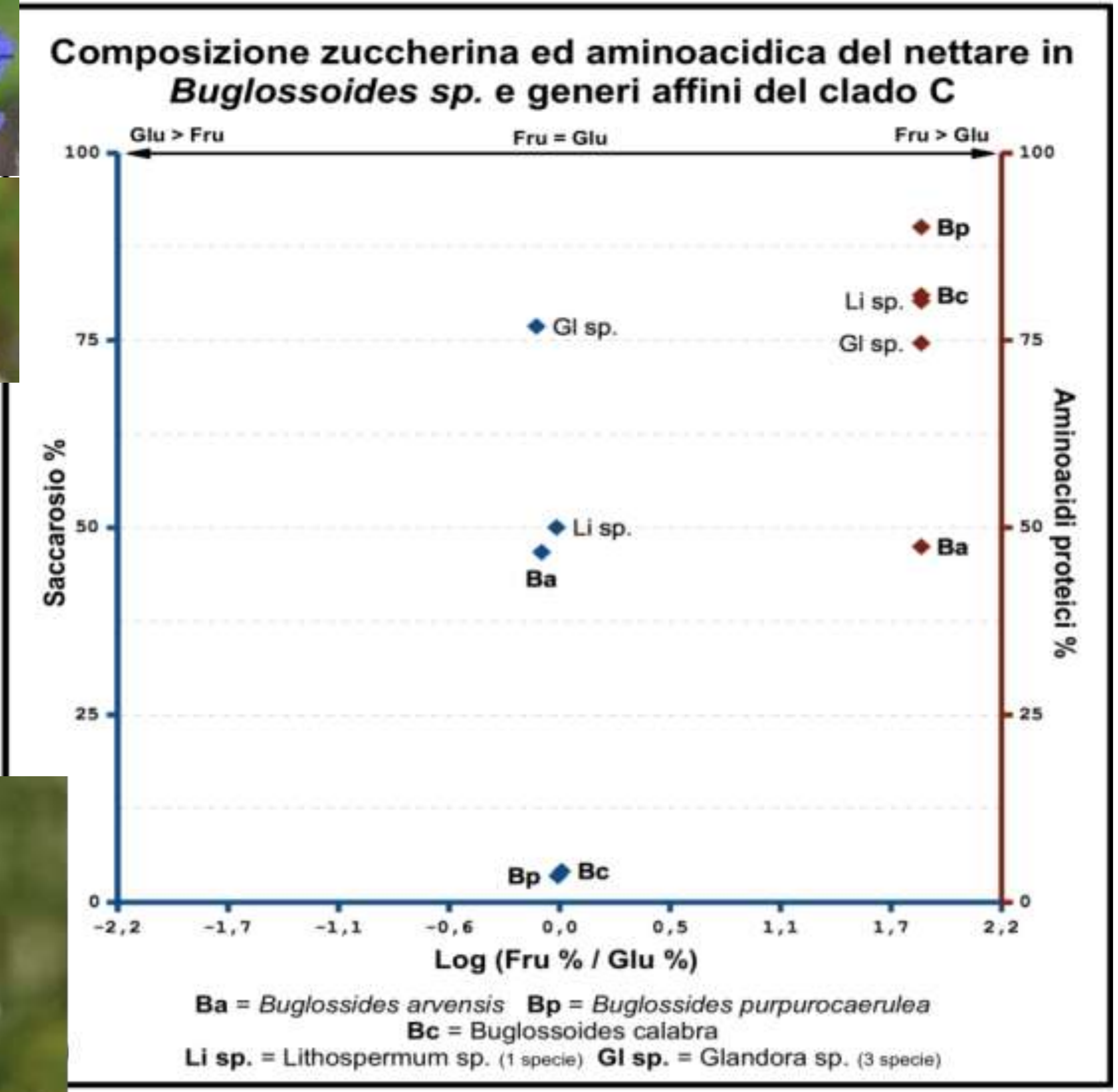
β-alanine,
γ-aminobutyric acid (GABA),
taurine

Phenotipic plasticity of nectar composition



High plasticity of nectar traits





Amino acid	Total AA%
Pro	56.18
Ala	10.91
Ser	10.07
β-Ala	4.92
GABA	3.82

Amino acid	Total AA%
Pro	25.44
Ala	10.24
Ser	3.25
β-Ala	9.29
GABA	33.13



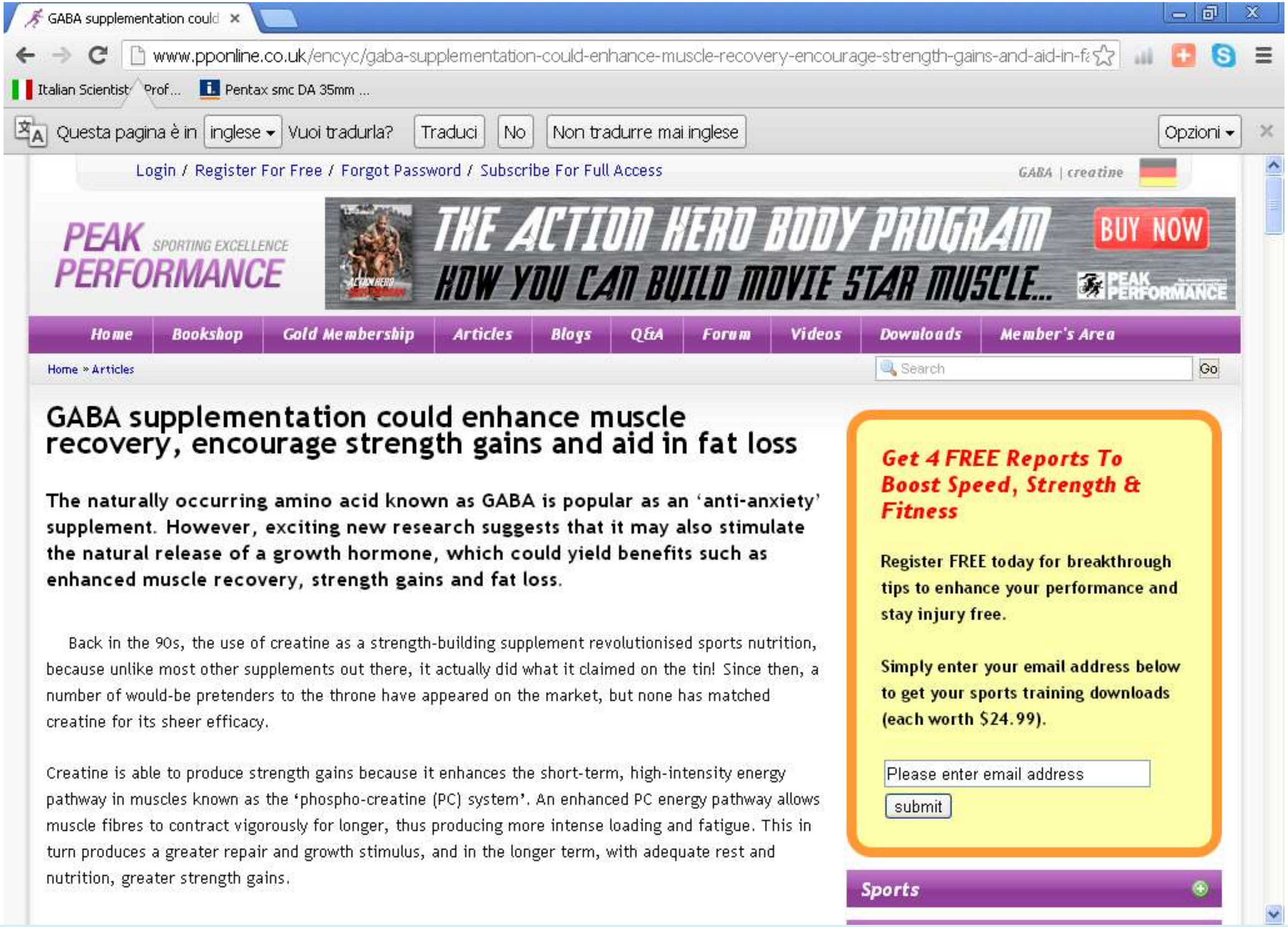


Aloe sp.


Constant nectar composition despite the large number of different pollinators

Strong
Phylogenetic constrain

What are nectar NPAAs for?



GABA supplementation could enhance muscle recovery, encourage strength gains and aid in fat loss

The naturally occurring amino acid known as GABA is popular as an 'anti-anxiety' supplement. However, exciting new research suggests that it may also stimulate the natural release of a growth hormone, which could yield benefits such as enhanced muscle recovery, strength gains and fat loss.

Back in the 90s, the use of creatine as a strength-building supplement revolutionised sports nutrition, because unlike most other supplements out there, it actually did what it claimed on the tin! Since then, a number of would-be pretenders to the throne have appeared on the market, but none has matched creatine for its sheer efficacy.

Creatine is able to produce strength gains because it enhances the short-term, high-intensity energy pathway in muscles known as the 'phospho-creatine (PC) system'. An enhanced PC energy pathway allows muscle fibres to contract vigorously for longer, thus producing more intense loading and fatigue. This in turn produces a greater repair and growth stimulus, and in the longer term, with adequate rest and nutrition, greater strength gains.

Nectar NPAAs

A supplement for muscular activity in the nectar?

Natural doping for insects!!

+ insect flying ability = + possibility of pollination

Journal of Ecology — BRITISH ECOLOGICAL SOCIETY

Future Directions No. 9 | Free Access | *Journal of Ecology* 2014, **102**, 108–115

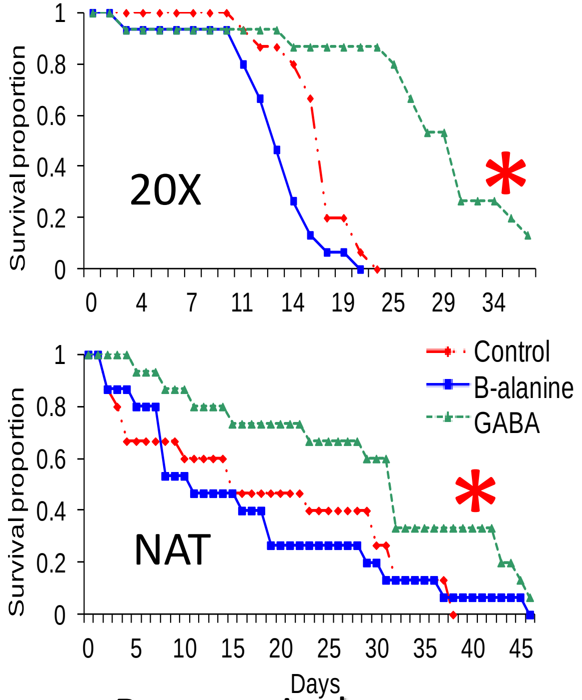
Beyond nectar sweetness: the hidden ecological role of non-protein amino acids in nectar

Massimo Nepi

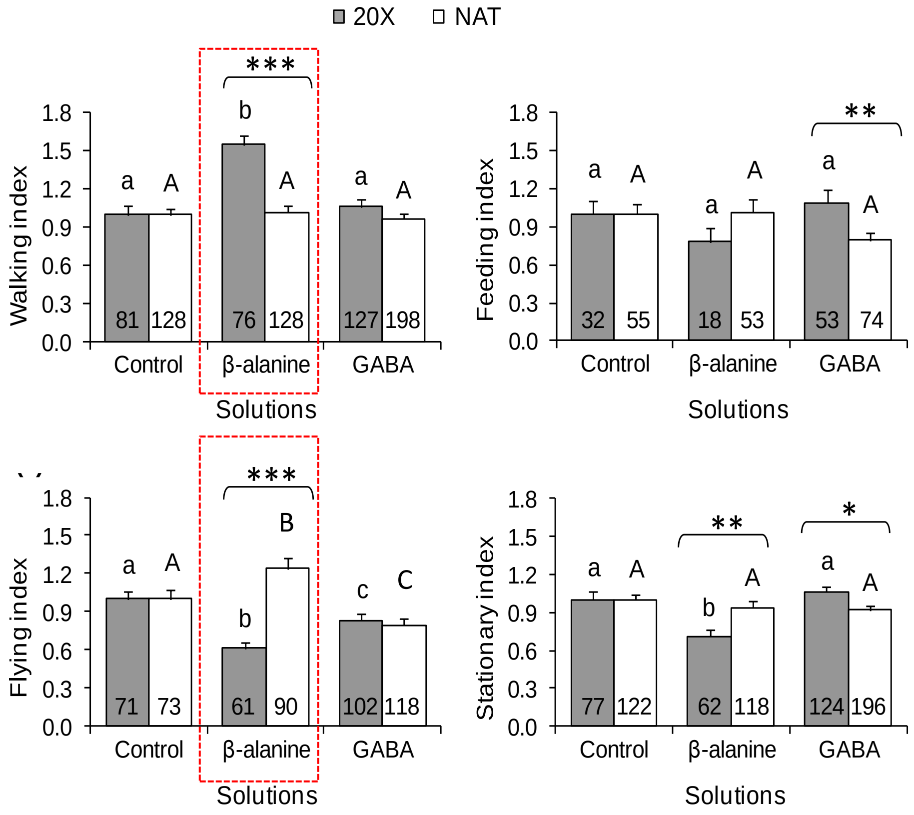
Effects of Non-Protein Amino Acids in Nectar on Bee Survival and Behavior

Gherardo Bogo, Laura Bortolotti, Simona Sagona, Antonio Felicioli, Marta Galloni, Marta Barberis & Massimo Nepi

Journal of Chemical Ecology 45, 278–285(2019) | Cite this article



Bee survival

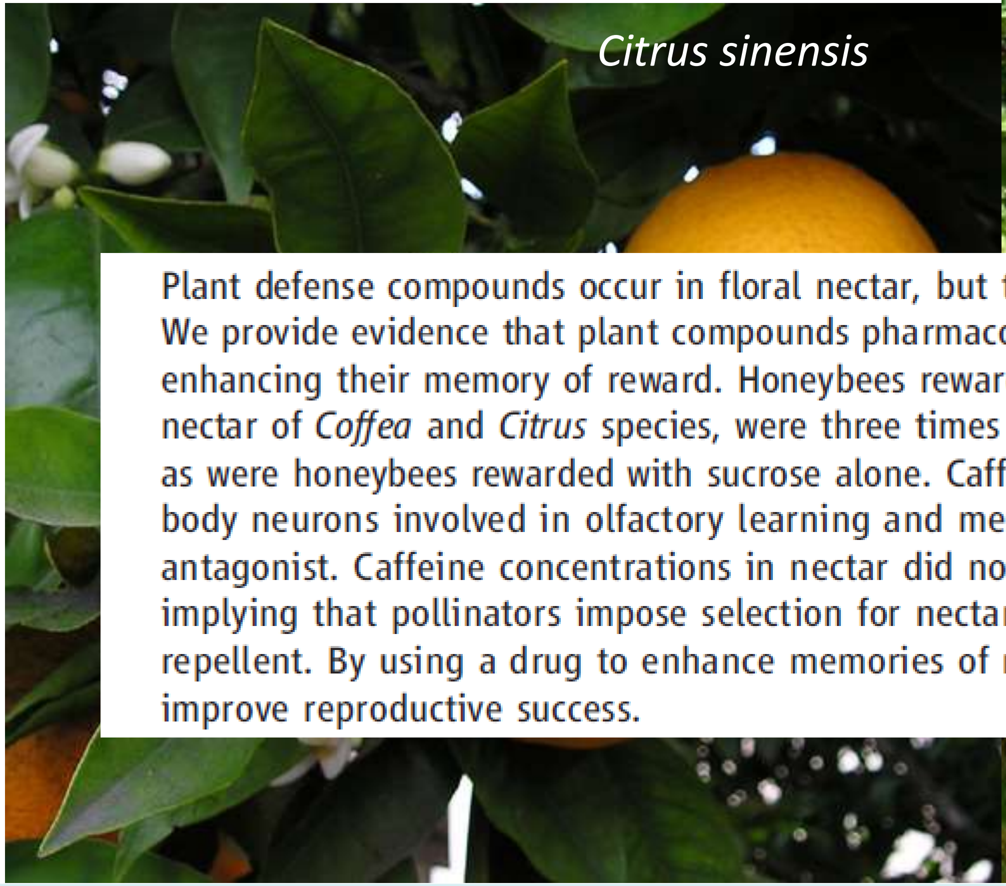


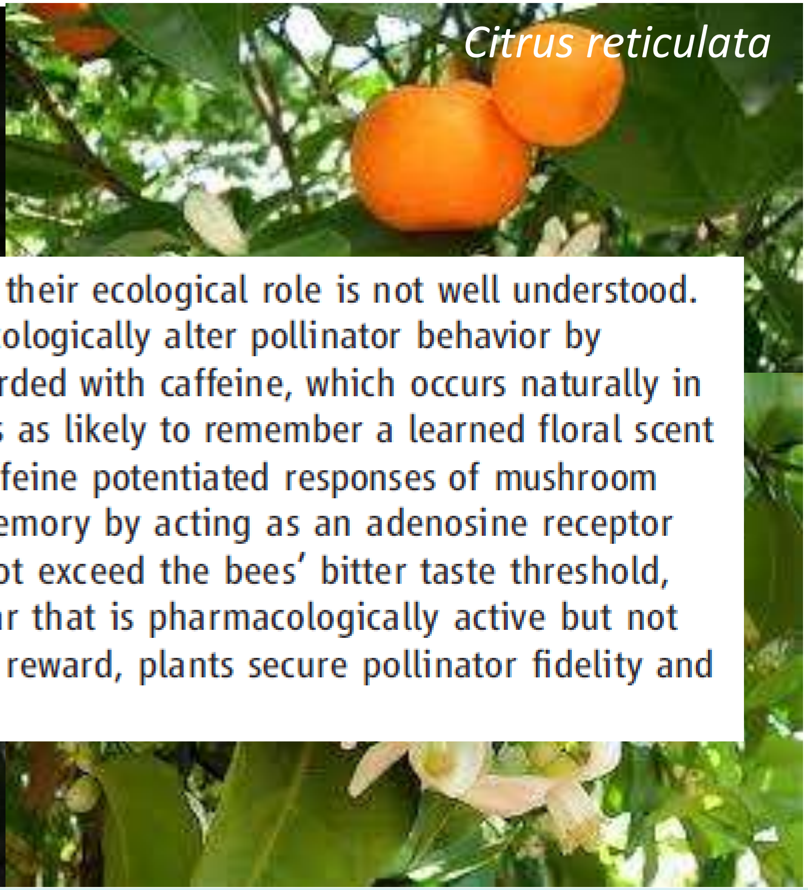
Behavioural observations

ALKALOIDS IN NECATR

Science AAAS

Caffeine in Floral Nectar Enhances a Pollinator's Memory of Reward
G. A. Wright *et al.*
Science **339**, 1202 (2013);
DOI: 10.1126/science.1228806





Plant defense compounds occur in floral nectar, but their ecological role is not well understood. We provide evidence that plant compounds pharmacologically alter pollinator behavior by enhancing their memory of reward. Honeybees rewarded with caffeine, which occurs naturally in nectar of *Coffea* and *Citrus* species, were three times as likely to remember a learned floral scent as were honeybees rewarded with sucrose alone. Caffeine potentiated responses of mushroom body neurons involved in olfactory learning and memory by acting as an adenosine receptor antagonist. Caffeine concentrations in nectar did not exceed the bees' bitter taste threshold, implying that pollinators impose selection for nectar that is pharmacologically active but not repellent. By using a drug to enhance memories of reward, plants secure pollinator fidelity and improve reproductive success.

SCIENTIFIC REPORTS

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Nicotine in floral nectar pharmacologically influences bumblebee learning of floral features

D. Baracchi[1,3], A. Marples[2], A. J. Jenkins[1], A. R. Leitch[1] & L. Chittka[1]

Many plants defend themselves against herbivores by chemical deterrents in their tissues and the presence of such substances in floral nectar means that pollinators often encounter them when foraging. The effect of such substances on the foraging behaviour of pollinators is poorly understood. Using artificial flowers in tightly-controlled laboratory settings, we examined the effects of the alkaloid nicotine on bumblebee foraging performance. We found that bumblebees confronted simultaneously with two equally rewarded nicotine-containing and nicotine-free flower types are deterred only by unnaturally high nicotine concentrations. This deterrence disappears or even turns into attraction at lower nectar-relevant concentrations. The alkaloid has profound effects on learning in a dose-dependent manner. At a high natural dose, bees learn the colour of a nicotine-containing flower type more swiftly than a flower type with the same caloric value but without nicotine. Furthermore, after experiencing flowers containing nicotine in any tested concentration, increasing numbers of bumblebees stay more faithful to these flowers, even if they become a suboptimal choice in terms of reward. These results demonstrate that alkaloids enhance pollinator flower constancy, opening new perspectives in co-evolutionary process between plants and pollinators.

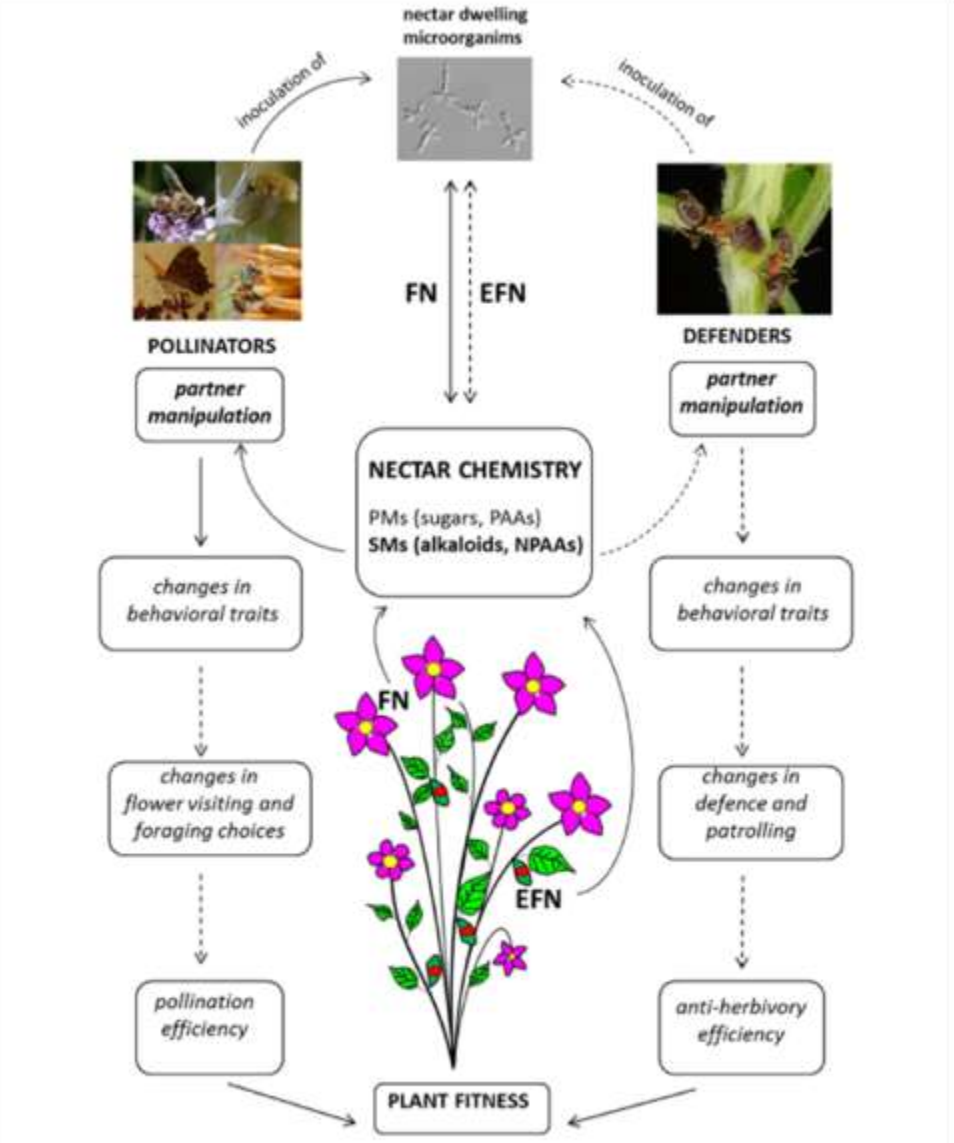

FIGURE 2 | Diagram of nectar-mediated manipulation of pollinators and tending ants. Full lines indicate processes/interactions sustained by scientific evidence; hatched lines indicate processes/interactions for which scientific evidence is not yet available. FN, floral nectar; EFN, extra-floral nectar; PMs, primary metabolites; PAAs, protein amino acids; NPAAs, non-protein amino acids; SMs, secondary metabolites. Picture of nectar-dwelling microorganism (*Metschnikowia gruessii*) reproduced with permission from Carlos M. Herrera.

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Front. Plant Sci., 19 July 2018 | https://doi.org/10.3389/fpls.2018.01063

frontiers in Plant Science

Nectar in Plant–Insect Mutualistic Relationships: From Food Reward to Partner Manipulation

Massimo Nepi[1*], Donato A. Grasso[2] and Stefano Mancuso[3]

Floral nectar is much more than simply a food reward for pollinators!!!

THANK YOU FOR YOUR KIND ATTENTION