Is main sugar composition of floral nectar determined by pollinators in *Scrophularia* species?

Rodríguez-Riaño T.\(^1\), Ortega-Olivencia A.\(^1\), López J.\(^1\), Pérez-Bote J.L.\(^2\), Navarro-Pérez M.L.\(^1\), Valtueña F.J.\(^1\) & Mayo C.

\(^1\) University of Extremadura, Faculty of Sciences, Area of Botany, Badajoz (Spain)

\(^2\) University of Extremadura, Faculty of Sciences, Area of Zoology, Badajoz (Spain)
Pollination syndromes
Plant pollinators can be inferred from a suite of floral characters, including nectar (Faegri & van der Pijl 1979)
Demonstrated convergence between nectar sugar composition and pollination syndromes (Baker & Baker 1983; Nicholson & Thorburg 2007)

Main trends indicated that

- **Hummingbirds, butterflies, moths and long-tongued bees**
  - Nectar ➔ Sucrose

- **Passerines, flies, bats and short-tongued bees**
  - Nectar ➔ Hexoses
Same pollinators (pollination syndrome) exhibiting similar nectar traits

Plant phylogeny stronger determinant of nectar chemistry than pollinator

Taxonomically determined

Climatic factors or geographical distribution imposed nectar sugar uniformity

Mediterranean region (prolonged summer drought)
Scrophularia

Strongly supported monophyletic (Navarro-Pérez et al. 2013) and mostly Holartic genus with two recognised sections and aprox. 270 sp (Stiefelhagen 1910, Ortega-Olivencia 2009):

**Sect. Scrophularia**
Herbs with abundant leaf development

**Sect. Caninae**
Semi-shrubby and xerophyte with poor leaf development
Hermaphrodite protogynous flowers, with tubular-urceolate and two-lipped corolla, four stamens adnate to the lower lip of the corolla, one staminode, bicarpellar gynoecium and an annular nectary.

*Scrophularia*

Staminode

Nectary

♂ phase

♀ phase
Broad discrimination of European *Scrophularia* into 3 groups relating to their corolla size (vide Ortega-Olivencia 2009)

**Small and violet flowers**
Expectance: pollinated by hoverflies with high-hexoses nectar
(Baker & Baker 1983; Petanidou 2005)

**Medium-sized and dull-coloured flowers**
(purple, brown or yellowish-green)
Expectance: pollinated by wasps with high-sucrose nectar
(Baker & Baker 1983; Petanidou 2005; Nocentini et al. 2013)

**Large and showy flowers**
Expectance: some bird interaction with high-hexoses nectar
Aims of this study

To study in three *Scrophularia* species groups the following aspects:

i. Pollination system vs. pollination syndromes

ii. Nectar production and nectar sugar composition

iii. Association between nectar sugar composition and pollinators

Our ultimate goal was:

- Phylogenetic constraints
- Nectar sugar composition
- Other factors (climatic, geographical)
Species studied

Small and violet flowers

Sect. *Caninae*

- **S. canina**
  - Flower size
  - $3.55 \pm 0.51 \text{ mm}$

- **S. frutescens**
  - Flower size
  - $3.86 \pm 0.48 \text{ mm}$

Studied populations
- Small point = 1 population
- Big point = at least two populations
Species studied

Medium-sized and dull-coloured flowers

Studied populations
Big point = at least two populations

Sect. *Scrophularia*

- **S. lyrata**
  - Flower size: $5.44 \pm 0.92$ mm

- **S. scorodonia**
  - Flower size: $6.80 \pm 0.57$ mm
Sect. *Scrophularia*

**Large and showy flowers**

**Species studied**

*Studied populations*

Big point = at least two populations

- **S. grandiflora**
  - Flower size: $13.23 \pm 2.32$ mm

- **S. sambucifolia**
  - Flower size: $11.78 \pm 1.97$ mm
Sect. *Scrophularia*

Large and showy flowers

Species studied

**Studied populations**
- Small point = 1 population
- Big point = at least two populations

**S. calliantha**
- Flower size: $15.99 \pm 2.28$ mm

**S. trifoliata**
- Flower size: $11.98 \pm 1.81$ mm
Nectar measures

Two inflorescences (individuals)/population; eight flowers/individual (4 flowers/sexual phase)
Day before analysis inflorescence were bagged. On the following day nectar traits were measured:

**Volume:** graduated micropipettes

**Concentration:** manual refractometers

**Nectar sugar composition:** HPLC (high pressure liquid chromatography)

Small aliquot of nectar was spotted onto Whatman No. 1 paper and frozen in a Petri dish for subsequent analysis
Pollination system

Pollinator censuses in natural populations during peak flowering
Numerous 10 min periods during daylights
Behavior and reward sought by each floral visitor
Pollinator: contacting both stamens and stigmas during their visits

Pollinators were classified into seven functional groups according to their size, taxonomy and/or behavior while visiting *Scrophularia* flowers

- Bumblebee (Apinae)
- Hoverfly (Syrphidae)
- Small bee and small wasp (Andrenidae, Anthophoridae, Eumeninae, Halictidae, Megachilidae)
- Bird (Passeriformes)
- Honeybee (Apinae)
- Wasp (Vespinae)
- Lizard (Squamata)

Visitation rate was used to measure pollinator importance
### Dipteran pollination system

**Small and violet flowers**

<table>
<thead>
<tr>
<th>N. of censuses (10 m)</th>
<th>Rab</th>
<th>Alor</th>
<th>Roch</th>
<th>Port</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hoverflies</strong></td>
<td>65</td>
<td>145</td>
<td>17</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>0.053</td>
<td>0.254</td>
<td>0.085</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>Small bees and wasps</strong></td>
<td>0.015</td>
<td>0.011</td>
<td>0.105</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Wasps</strong></td>
<td>0.015</td>
<td>0.011</td>
<td>0.105</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Honeybees</strong></td>
<td>0.001</td>
<td>0.005</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Bumblebees</strong></td>
<td>0.001</td>
<td>0.005</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Passerine birds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Lizards</strong></td>
<td></td>
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</tr>
</tbody>
</table>

**S. canina** (small bee) | **S. frutescens** (hoverfly)
Medium-sized and dull-coloured flowers

Wasp pollination system

S. scorodonia (small bee)

<table>
<thead>
<tr>
<th>Visitation rates</th>
<th>S. lyrata</th>
<th>S. scorodonia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bada</td>
<td>Oliv</td>
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<tr>
<td>N. of censuses (10 m)</td>
<td>50</td>
<td>107</td>
</tr>
<tr>
<td>Hoverflies</td>
<td>0.005</td>
<td>0.011</td>
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<tr>
<td>Small bees and wasps</td>
<td>0.072</td>
<td>0.013</td>
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<tr>
<td>Wasps</td>
<td><strong>0.199</strong></td>
<td>0.250</td>
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<tr>
<td>Honeybees</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>Bumblebees</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Passerine birds</td>
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<td></td>
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<tr>
<td>Lizards</td>
<td></td>
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</tbody>
</table>
Large and showy flowers

Mixed vertebrate-insect pollination system

Prevalence of insects

<table>
<thead>
<tr>
<th></th>
<th>Visitation rates</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>S. grandiflora</strong></td>
<td><strong>S. sambucifolia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N. of censuses (10 m)</strong></td>
<td>Rab</td>
<td>Pomb</td>
<td>Gast</td>
<td>Mor</td>
<td></td>
</tr>
<tr>
<td>Hoverflies</td>
<td>692(134)</td>
<td>381(32)</td>
<td>439(134)</td>
<td>409(114)</td>
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</tr>
<tr>
<td>Small bees and wasps</td>
<td>0.029</td>
<td>0.029</td>
<td>0.023</td>
<td>0.013</td>
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<tr>
<td>Wasps</td>
<td>0.078</td>
<td>0.157</td>
<td>0.047</td>
<td>0.029</td>
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<tr>
<td>Honeybees</td>
<td>0.069</td>
<td>0.010</td>
<td>0.108</td>
<td>0.440</td>
<td></td>
</tr>
<tr>
<td>Bumblebees</td>
<td><strong>0.484</strong></td>
<td><strong>0.754</strong></td>
<td>0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passerine birds</td>
<td>0.092</td>
<td>0.033</td>
<td>0.039</td>
<td>0.018</td>
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</tr>
<tr>
<td>Lizards</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*S. sambucifolia* (honeybee)

*S. grandiflora* (bumblebee)
Large and showy flowers

Mixed vertebrate-insect pollination system with prevalence of insects

<table>
<thead>
<tr>
<th>Visitation rates</th>
<th>S. trifoliata</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. of censuses (10 m)</td>
<td>41(26)</td>
</tr>
<tr>
<td>Hoverflies</td>
<td></td>
</tr>
<tr>
<td>Small bees and wasps</td>
<td></td>
</tr>
<tr>
<td>Wasps</td>
<td>0.142</td>
</tr>
<tr>
<td>Honeybees</td>
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<td>Lizards</td>
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</tbody>
</table>

Wasp (Vespula)

Blackcap (Sylvia atricapilla)
Large and showy flowers

Mixed vertebrate-insect pollination system

Prevalence of birds

<table>
<thead>
<tr>
<th>Visitation rates</th>
</tr>
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<tbody>
<tr>
<td><strong>S. calliantha</strong></td>
</tr>
<tr>
<td>N. of censuses (10 m)</td>
</tr>
<tr>
<td>Hoverflies</td>
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<tr>
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</tr>
<tr>
<td>Passerine birds</td>
</tr>
<tr>
<td>Lizards</td>
</tr>
</tbody>
</table>

Canarian chiffchaff (*Phylloscopus canariensis*)

Small bee
MVI = Mixed vertebrate-insect pollination system

\[ F = 66.08^{***} \]

\[ F = 6.28^{**} \]

\[ F = 25.30^{***} \]

\[ F = 27.37^{**} \]

\[ F = 12.43^{***} \]

MVI = Mixed vertebrate-insect pollination system
Balanced nectar = nectar containing equal weights of sucrose and hexoses, i.e. when sugar ratio is equal or close to one.
Pollination systems observed in *Scrophularia* species are the following:

i. Hoverflies together with small bees for small-sized flower species with high-sucrose nectar

ii. Wasps (sometimes accompanied by bumblebees) for medium-sized flower species with high-sucrose nectar

iii. Mixed vertebrate-insect with prevalence of insects (wasp, honeybee or bumblebee) for the Mediterranean group of large flowers with high-sucrose nectar

iv. Mixed vertebrate-insect with prevalence of passerine birds for the Macaronesian group of large flowers (*S. calliantha*) with balanced nectar

**In summary**
Scrophularia species have different pollination systems.

Other species of Scrophularia

Medium-sized flowered species with wasp as principal pollinator:
- *S. nodosa* (Percival 1961);
- *S. smithii* (Dupont et al. 2004);
- *S. umbrosa* (Brodmann et al. 2012)

Small-sized flowered species with unknown pollination system:
- *S. vernalis* (Percival 1961)

Similar nectar sugar composition
- Sucrose

Except *S. calliantha*
- Balanced nectar

Nectar sugar composition flows into Pollination system:
- Sucrose nectar
Nectar sugar composition  X  Pollination system
Nectar sugar composition virtually uniform

Climatic or geographical factors

Mediterranean region

Petanidou 2005
High-sucrose nectar
High-hexoses nectar

Examined species

Mediterranean distribution

High-sucrose nectar
Balanced nectar
(S. calliantha)

no Mediterranean (Macaronesian)

Other Scrophularia (not subject to a typical Mediterranean climate)

S. nodosa (temperate Eurasia)
S. vernalis (mostly temperate Europe)
S. umbrosa (temperate Eurasia)

S. smithii (endemic to Canary Islands)
Nectar sugar composition virtually uniform

X

Climatic or geographical factors
Nectar sugar composition

Pollination system

Climatic or geographical factors

Nectar sugar composition near uniformity

Conservative taxonomic character
Additional *Scrophularia* species

- *S. vernalis* (high sucrose nectar)
- *S. nodosa* (high sucrose nectar)
- *S. umbrosa* (high sucrose nectar)

Mediterranean clade

- *S. grandiflora* (high sucrose nectar)
- *S. sambucifolia* (high sucrose nectar)
- *S. trifoliata* (high sucrose nectar)

**Sect. Caninae**

- *S. canina* (high sucrose nectar)
- *S. frutescens* (high sucrose nectar)

(Nearest relational clade)

**Rest of Sect. *Scrophularia***

Macaronesian subclade

- *S. calliantha* (balanced nectar)
- *S. smithii* (high sucrose nectar)

(Meditteranean clade)

- *S. grandiflora* (high sucrose nectar)
- *S. sambucifolia* (high sucrose nectar)
- *S. trifoliata* (high sucrose nectar)

**Sect. Caninae**

- *S. canina* (high sucrose nectar)
- *S. frutescens* (high sucrose nectar)

(modified from Navarro-Pérez et al., 2013)
Sugar composition in this Euro-Macaronesian sample of *Scrophularia* may be a *conservative trait* resulting from phylogenetic constraints.
Thank you very much

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