Comparative floral traits in Corunastylis (Diurideae; Orchidaceae) with novel applications: do some species bleed or blink?

Zong-Xin Ren¹, Wendy Grimm², Brian Towle³, Qi Qiao⁴, Peter Bernhardt⁵

¹ CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Yunnan 650201, People’s Republic of China
² Department of Biological Sciences, Macquarie University, New South Wales 2109, Australia
³ Ecoplanning Pty Ltd, 74 Hutton Avenue, Bulli, New South Wales 2516, Australia
⁴ College of Agriculture, Henan University of Science and Technology, Luoyang, Henan 471000, China
⁵ Department of Biology, Saint Louis University, St. Louis, Missouri 63103, USA; e-mail: bernhap2@slu.edu

Abstract

We compared suites of inflorescence and floral traits of six taxa in the genus Corunastylis. Liquid rewards were not detected at the bases of labellum calluses in three species. Instead, glabrous auricle lobes containing variable numbers of raphides secreted droplets. Scent analyses identified seven compounds in three species, with five for C. ruppii, sharing 8-heptadecene with C. filiformis. A previous hypothesis that these flowers mimic wounded insects offering “mock haemolymph” overlaps with the suggestion here that scents and trembling labella mimic blinking, weeping eyes.

Keywords: Corunastylis, auricles, labellum, mimicry, raphides, staminodes

Introduction

True flies (Diptera) are the primary pollinators of many species within the family Orchidaceae (Dressler 1993; Larson et al. 2001). However, some fly pollinators are < 3 mm in length. These micro-dipterans belong to members of the families Ceratopogonidae, Chloropidae, Culicidae, Drosophilidae, Milichiidae, Mycetophilidae, Phoridae, Scatopsidae and Sciaridae (Thien 1969, Larson et al. 2001; Kuiter 2016). Many Neotropical orchids in the subtribe Pleurothallidinae (Epidendroideae; sensu Neyland et al 1995) are pollinated by micro-dipterans in different families. Floral variation within pleurothallid lineages indicates complex biochemical and morphological modifications in the evolution of attractants, rewards and/or suites of traits indicative of sexual mimesis (Borba & Semir 2001; Blanco & Barboza 2005; Karremans et al. 2015; Bogarin et al. 2018).

Australia is also a centre of orchid pollination by micro-dipterans. Weston et al. (2014) reviewed floral evolution in indigenous species in the tribe Diurideae and found that some species in the genera Acianthus,
Corybas (as Corysanthes s.s. and Singularybas) and Rhizanthella (sensu Jones 2006) required pollinators belonging to one to four families containing micro-dipterans. In addition, members of the Australasian genus Pterostylis (Cranichideae) and its allies were pollinated by small flies in the Mycetophilidae and Scaridae (Gaskett 2011; Kuiter 2016). Most of these Australasian species bloom only in the temperate, Australian winter into mid-spring (Jones 2006).

The Australasian genus Corunastylis (sensu Clements & Jones 2002; Clements et al. 2002; Jones et al. 2002) contains over 50 species (Jones 2006; Jones & Clements 2018) and is also dependent on micro-dipterans. However, micro-dipteran pollination in Corunastylis species appears to differ from the other Australasian genera in five ways. First, the majority of Corunastylis species bloom from summer into mid-autumn. Second, they present non-resupinate flowers. Third, they may be the only taxa in temperate Australia to be pollinated primarily by members of the Chloropidae and, to a lesser extent, by the Millichidae and Scatopsidae (Weston et al. 2014; Bower et al. 2015; Kuiter 2016, 2018). Fourth, the flowers of many Corunastylis species have a floral organ that Garnet (1940) referred to as the “comically tremulous labellum.” That is, the labellum is attached to a column foot by such a thin hinge that it flaps or trembles in the slightest breeze. Fifth, while most Corunastylis species present labellum surfaces that show dark pigmentation, calluses, and are bearded-ciliate, there is no evidence that they mimic brood-sites, edible resources or sexually receptive females. Floral scents, when recorded at all, were described previously as lemon-scented in four species (Blaxell 1970; Jones 2006), musty and unpleasant for C. bishopii (D.L.Jones & M.A.Clem.) D.L.Jones & M.A.Clem. (syn. Genoplesium) also found pollinators in two genera in the Chloropidae (Conioscinella and Cadrema). They photographed nectar droplets in the grooves of the labellum callus. Although most chloropid specimens bearing pollinaria were females, the authors did not interpret C. littoralis as a brood-site mimic due to the absence of eggs in the flowers and the lack of discernible dung or carrion-like scents. However, many members in the Chloropidae do not oviposit in dung or corpses. Instead, the maggots of some species consume a wide variety of plant or animal resources (Arnett 1985).

Literature on the role of chloropids as pollinators remains uncommon ( Larson et al. 2001; Oelschlagel et al. 2015; Kuiter 2016; Wiesenborn 2016). In particular, there is a lack of information regarding intra- and interspecific variation in traits offering attractants and rewards to chloropids visiting flowers of Corunastylis species. This includes the investigation and clarification of the number of flowers per inflorescence (display), histology, scent analyses and the prospective location of rewards in understudied species. Our results will be used to propose a novel hypothesis for pollination syndromes in some members of this genus.

Methods

Study sites, observation and collection dates

Flowers of Corunastylis were observed and collected in New South Wales and Victoria. To protect extant populations from poaching, GPS coordinates are withheld following the Sensitive Species Data Policy in New South Wales ( Andrews 2009) along with detailed descriptions of vegetation. We sampled the following sites containing the following species.


3. NEW SOUTH WALES. Kulnura. Collection of inflorescences of *C. filiformis* and *C. ruppii* (R.S.Rogers) D.L.Jones & M.A.Clem (05/02–18/03/2016) along graded, roadside verges bordering sclerophyll shrublands and farms.

4. NEW SOUTH WALES. Royal National Park. Collection of inflorescences of *C. filiformis* and *C. rufa* (R.Br.) D.L.Jones & M.A.Clem (19/02/2016) along the margins of a fire trail in a slashed powerline easement through shrubby sub-formation of dry sclerophyll forest.

5. NEW SOUTH WALES. Heathcote National Park. Collection of *C. filiformis* (Fitzg.) D.L.Jones & M.A.Clem (11/03/2017) in dry, sclerophyll forest with coastal sandstone ridgetop woodland community. Specimens were collected only for additional microscopy on column wing histology (see Results and Discussion).


Comparative flowering periods and the number and development of flowers on inflorescences

Flowering periods for the populations from which collections were made were recorded from 2015–2016, and again in 2019 for *Corunastylis ruppii* at Kur-ring-gai Chase. Inflorescences were selected at random, and the number of flowers per inflorescence counted using 3× optical glass binocular magnifiers (Opti Visor, Donegan Optical Co.) or by placing whole, collected scapes under a dissecting microscope following fixation in 3:1 95% ethanol:glacial acetic acid. Patterns of floral development (open or closed) and the order of flower bud opening (synchronous, acropetal or basipetal) were recorded. Although it was estimated that the population of *C. ciliata* contained >300 scapes in 2016, all were in different stages of fruiting. To determine the original number of flowers on inflorescences in this species, the number of withered flowers, fruits and flower scars on each scape were also counted.

Labellum observations

The trembling of labella of open flowers in the wind was analysed in several species and one putative hybrid. This also included probing the labella of whole flowers on inflorescences under a dissecting microscope. Labellum calluses were examined for the presence of nectar droplets in open flowers of *Corunastylis filiformis*, *C. fimbriata* and *C. ruppii*, isolated under organza bags. To stabilise the slender scapes, a bamboo skewer was inserted into the earth next to each inflorescence while it was still in bud. The whole inflorescence and skewer were covered with an organza bag. The skewer served to prop up the bag so that the weight of the bag was never set upon the scape. The bag was removed to examine open flowers 24–48 hours following anthesis and after the evaporation of morning dew. Specimens were also collected of *C. fimbriata* and *C. ruppii* found severed and lying on the ground, presumably through damage by unknown animals. At field sites, buds and flowers were viewed while wearing an Opti Visor.

Secretion in column appendages

**Terminology.** Previous and unpublished observations, by W. Grimm on *Corunastylis fimbriata*, and on *C. filiformis* by B. Towle, suggested that the column appendages (*sensu* Nicholls 1969) of these species contained secretory structures. Each pair of column appendages in a *Corunastylis* flower is bilobed and the lobes are fused at their bases. They are often referred to as anterior and posterior lobes, but the terms were deemed inadequate and confusing in non-resupinate flowers. Therefore, the terminology of Kurzweil et al. (2005) was adopted, subdividing the two appendage lobes based on their divergent ontogenetic origins. The lobe closest to the fertile anther is referred to here as the auricle and the lobe beneath the auricle is referred to here as the staminode. Cell and tissue terminology in these lobes follows Fahn (1979).

**Observing and processing tissues.** In 2016, it was observed whether column appendages secreted droplets in *Corunastylis filiformis*, *C. fimbriata* and *C. ruppii* exposed to the air and protected under organza bags. To preserve specimens for lab microscopy, whole inflorescences were fixed in 3:1 95% ethanol:glacial acetic acid for 2–6 hours, then decanted and preserved...
in 70% ethanol. This treatment keeps floral organs flexible but also clears pigmentation to observe tissue layers, locate specific cells and count vascular strands. Whole column appendages were excised from their columns, mounted on glass slides with distilled water for light microscopy, or with decolourised aniline blue for epifluorescence (see Goldblatt & Bernhardt 1990). Cleared specimens were viewed and photographed either under a Zeiss Axioskop 40 (see Edens-Meier et al. 2010) or a Zeiss Axio Imager M2. We looked for the presence of raphides in specimens mounted in distilled water. When raphide cells are clustered in orchid organs they indicate sites of slime or mucilage production (Smith 1923) and floral secretions (Bogarin et al. 2018). As nectar sugars are often supplied by phloem, we mounted specimens in decolorised aniline blue (Fahn 1979; Croy 1993) contrasting specimens under both polarised light and epifluorescence to locate callose in sieve cells.

Scent collection and analyses

Floral scents of Corunastylis filiformis, C. fimbriata and C. ruppii were collected in situ as described by Edens-Meier et al. (2014). Four inflorescences of each species were selected at random. Each inflorescence had 4–12 open flowers, and diurnal scent collection was performed on plants at the Bloodwood Road, Kulnura and Ku-ring-gai Chase sites. A headspace bag (Reynolds® Oven Bag; Reynolds, Inc., Richmond, VA, USA) was cut to dimensions of 10 × 10 cm and was used to cover each inflorescence. The bag was sealed at the bottom using a twist tie. An adsorbent trap, prepared using a Pasteur pipette with 10 mg Porapak Q (80/100 mesh; SUPELCO, Bellefonte, PA, USA) packed between glass wool, was attached to a battery-operated PAS-500 vacuum pump (Spectrex, Inc.) with Tygon tubing. The terminus of the trap was then sealed within the top of the headspace bag with a second twist tie. Floral scent was collected for two hours in the morning from 09:00–11:00, avoiding full sun, from January to April 2016 at a standardised flow rate of 200 mL air/min. At each of the three sites ambient air controls were also taken to account for, and to later eliminate, non-floral compounds. Upon completion of the fragrance collection, scent traps were eluted into 1.5 mL borosilicate glass auto-sampler vials using 300 μl of GC-MS grade hexane. Each vial was capped, labelled, wrapped with parafilm, and stored at -20°C. All collected sample vials were sent to the Kunming Institute of Botany, Chinese Academy of Sciences for GC-MS analyses.

Floral headspace samples eluted in hexane were concentrated to 50 μl under a flow of nitrogen gas (N2). An internal standard of 5 μl of a 0.03% solution of tolune (23.6 ng) in hexane was added to each sample. The volatiles were analysed on a Hewlett-Packard HP 6890 Series GC System coupled to a Hewlett Packard 5973 Mass Selective Detector. An HP-5MS column (5% phenyl-methylpolysiloxane, 30 m long with an inner diameter of 0.25 mm and a film thickness of 0.25 μm; Agilent, USA) was used for analyses. Each 1 μl sample was injected at 240°C. Electronic flow control was used to maintain a constant helium gas flow of 1.0 mL/min. The GC oven temperature began at 40°C and increased 3°C per min to 80°C, then increased 5°C per min to 280°C and was held for 20 minutes. The MS interface was 250°C. The ion trap worked at 230°C. The mass spectra were taken at 70 eV (in EI mode) with a scanning speed of one per scan from m/z 35 to 500. Component identification was carried out using NIST 05 mass spectral database, and Wiley 7n.1.

Results

Floral phenology and floral development

As predicted, species were found in bloom during the austral summer (Corunastylis filiformis, C. rufa, C. fimbriata and C. ruppii) and/or from late summer until early April (C. archeri, C. ciliata and C. morrisii). The number of flowers per inflorescence appears to vary at interspecific and intraspecific levels (Table 1). Corunastylis filiformis, C. fimbriata and C. ruppii were more likely to produce >11 flowers/scape. The maximum number of flowers counted on a scape (n=34) was recorded in one specimen of C. fimbriata at the Ku-ring-gai Chase National Park site. Flowering patterns were sub-acropetal to asynchronous in all species. As the population of C. ciliata was fruiting when it was observed, it was not possible to observe its mode of floral development. In peduncles producing a mean of >11 flowers/scape, inflorescences showed open development with flowers becoming increasingly small towards the scape apices as in Prasophyllum (see Bernhardt & Rowe 1993). The terminal flower buds did not open in C. filiformis, C. fimbriata and C. ruppii.
Figure 1. Secretion of auricles in *C. filiformis* (A) by B. Towle, and *C. fimbriata* (B) by Zong-Xin Ren.
Table 1. Comparative number of flowers/inflorescence (all sites pooled) and flowering periods (2016, 2017).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>n</th>
<th>Mean (S.D.)</th>
<th>Range</th>
<th>Flowering months (2016, 2017)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. archeri</em></td>
<td>26</td>
<td>5.6 (2.4)</td>
<td>2-12</td>
<td>Mar.–Apr.</td>
</tr>
<tr>
<td><em>C. archeri × C. ciliata</em></td>
<td>11</td>
<td>4.8 (2.3)</td>
<td>2-9</td>
<td>Mar.–Apr.</td>
</tr>
<tr>
<td><em>C. ciliata</em></td>
<td>67</td>
<td>6.2 (2.9)</td>
<td>2-14</td>
<td>Mar.</td>
</tr>
<tr>
<td><em>C. filiformis</em></td>
<td>53</td>
<td>12.9 (4.5)</td>
<td>6-22</td>
<td>Jan.–Mar.</td>
</tr>
<tr>
<td><em>C. fimbriata</em></td>
<td>86</td>
<td>15.3 (5.3)</td>
<td>7-34</td>
<td>Dec.–Feb.</td>
</tr>
<tr>
<td><em>C. morrisii</em></td>
<td>7</td>
<td>5.6 (4.4)</td>
<td>3-15</td>
<td>Mar.</td>
</tr>
<tr>
<td><em>C. ruppii</em></td>
<td>63</td>
<td>14.6 (6.0)</td>
<td>5-32</td>
<td>Jan.–Mar.</td>
</tr>
</tbody>
</table>

One Heathcote specimen of *C. filiformis* (collected 04/03/2017) produced seven, terminal, unopened buds.

**Labellum movement and secretions**

All species and the putative hybrid had movable, hinged labella. The labella of four species trembled with passing air currents (Table 2). The movement of *Corunastylis fimbriata* was recorded (see https://perma.cc/4FWF-548B and view live page). Two species were found not to tremble in passing air currents (Table 2) and this lack of movement *in situ* is recorded for *C. ruppii* (see https://perma.cc/M5VU-WVNR). However, the labella of both species did vibrate or tremble when the scape was tapped gently with a probe at field sites, or, when fresh flowers still attached to severed scapes were probed under a dissecting microscope (Table 2).

We did not find droplets at the bases of the labellum callus in *Corunastylis filiformis*, *C. fimbriata* or *C. ruppii* in bagged or open flowers. Additional observations and macrophotography in February and March 2020 by R. Kuiter (pers. comm.) showed nectar at the bases of the callus plates of *C. archeri*, *C. ciliata* and *C. morrisii* in Victoria, similar to descriptions by Garnet (1940).

**Column appendage secretions**

Secretions were not detected on any of the staminode lobes in any species. Droplets were first observed on the auricles in unbagged flowers of *Corunastylis filiformis* (Figure 1A) and *C. fimbriata* (Figure 1B) respectively in 2015 and 2016. Removal of the viscous fluid with microcapillary tubes showed that auricles of *C. fimbriata* produced less than 1 µl of fluid. No sweet taste was detected in secretions of *C. fimbriata* or *C. filiformis*. Bagged flowers of *C. ruppii* at Ku-ring-gai Chase National Park, did not produce visible droplets but a small quantity of viscous liquid was found on shiny auricles under 40× magnification under a binocular dissecting microscope. This fluid attached to a probe or dried to a crust on the auricle. A chloropid fly with a dorsal deposition of one pollinarium was seen and photographed as it regurgitated a droplet of fluid while perched on an opening flower of *C. fimbriata* at the Bloodwood Road site on 26/01/2016 (Figure 2). As the four remaining taxa were not bagged as part of this study, we are unable to confirm column appendage secretions in them.

**Floral epidermis and gross cytology of column appendages**

Each column appendage in each species contained one vascular trace under epifluorescence (Figure 3A). The trace is median to the point where the staminode...
is fused to the auricle, but it does not penetrate the apices of either of the lobes. In all species, staminodia are ornamented with elongated, unicellular trichomes, as compared to the almost glabrous auricles (Figure 3A). In Corunastylis morrisii and C. rufa the staminodia are longer than the auricles. In the remaining species, and the putative hybrid, the auricles are equal or sub-equal to the length of the staminodia (Figs. 3B–D). The apices of the auricle lobes of all species consisted of one to three tiers of large, overlapping cells containing granular cytoplasm. The marginal apex of the auricle of C. archeri (Figure 3A) and its hybrid, C. archeri × C. ciliata, is notched or a shallow bowl. The elongated auricle of C. fimbriata has a pore or depression at its apex (Figure 3B). The length of auricles and column appendages varied between inflorescences in the same species (Figures 3C and 3D).

Raphides were usually confined to the auricle lobes (Table 2, Figure 3C) but the numbers of raphides in each auricle varied between species, between members of the same species (Figure 3 C, D) and between flowers on the same inflorescence. Some staminode lobes in Corunastylis fimbriata contained a maximum of nine raphides in the auricles and 0–4 in the staminode lobe of the same column appendage (Table 2). In C. fimbriata and C. filiformis, raphides may be so congested at auricle apices that they overlap, are difficult to count and may turn the auricle apex black or brown as it dehydrates with age (Figure 3B–D). Observations of auricles from C. filiformis collected at Heathcote National Park retained 2–17 raphides (n=5 inflorescences) at their apices (Figures 3C and 3D). In the auricle of a single specimen of C. filiformis there was a maximum of 42 raphides with 14 raphides congested at the auricle apex and an additional four in the staminode lobe.

### Table 2. Raphides and labellum movement in Corunastylis taxa.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Range of raphides in auricles</th>
<th>Raphides in staminodes</th>
<th>Labellum trembles</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. archeri</td>
<td>4–16</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C. archeri × C. ciliata</td>
<td>1–10</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>C. filiformis</td>
<td>9–42</td>
<td>+ -</td>
<td>+</td>
</tr>
<tr>
<td>C. fimbriata</td>
<td>1–9</td>
<td>+ -</td>
<td>+</td>
</tr>
<tr>
<td>C. morrisii</td>
<td>0–15</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C. rufa</td>
<td>0–11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. ruppii</td>
<td>6–23</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Scent production and analysis

A lemony scent reminiscent of commercial extracts of rhizomes of Cymbopogon citratus (DC.) Stapf. was detected from fresh flowers of Corunastylis fimbriata, as described in the literature. However, on 15/01/16, three inflorescences of this species were placed into a clean, capped jar for 30 minutes. The accumulated odour was unpleasant, with a note reminiscent of a chlorinated swimming pool but dominated by “butcher shop smells” associated with oxidising fat on commercial cuts of lamb or beef. It was not possible to discern fragrances in the two remaining species even when they were placed in clean, capped jars. Scent analyses of C. filiformis, C. fimbriata and C. ruppii are based on retention times of 22.03–38.28 minutes. Corunastylis ruppii produced the greatest number of identifiable compounds. A total of 7 peaks recorded from 22.03–24.64 were found for C. fimbriata, but the long storage period (3 months) made it possible to identify only nepetalactone. Flowers of C. ruppii produced the greatest number of identifiable compounds (n=5) and we note that both C. ruppii and C. filiformis share 8-heptadecene, though at very different relative abundance (Table 3).

### Discussion

**General floral presentation**

The number of flowers produced by a raceme may vary between some Corunastylis species, with scapes of C. fimbriata producing the greatest number of flowers in 2016. While the labella of all Corunastylis species do not tremble in the wind, the labella of C. rufa and C. ruppii move under slight physical pressure.

Our observations of floral secretion and analyses of column appendage morphology strongly suggest
Figure 3. Column appendages of Corunastylis species. (A) Column wing of C. archeri showing the hairy staminodium lobe and the glabrous but notched auricle lobe (arrow). Note the phloem trace combining epifluorescence and polarized light by Qi Qiao; (B) Ageing column wing of C. fimbriata with dark, overlapping and congested raphides at apex in the elongated auricle and absence of raphides in the hairy staminode by Qi Qiao; (C) Raphides in the auricle and staminode lobes of C. filiformis (Heathcote National Park) by Peter Bernhardt; (D) Raphides in the auricle lobes of C. filiformis from a different flower from the same population (Heathcote National Park) by Peter Bernhardt. Note differences in length of the auricle lobes and distribution of raphides.

Table 3. Scent analyses of three Corunastylis species.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Highest relative abundance (%) recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. filiformis</td>
</tr>
<tr>
<td>Dodecen-1-ol</td>
<td>-</td>
</tr>
<tr>
<td>8-heptadecene</td>
<td>24.17</td>
</tr>
<tr>
<td>4-hydroxy-2-methylacetophenone</td>
<td>-</td>
</tr>
<tr>
<td>(4-isopropylphenyl)-methanol</td>
<td>-</td>
</tr>
<tr>
<td>Nepetalactone</td>
<td>-</td>
</tr>
<tr>
<td>Tridecane</td>
<td>-</td>
</tr>
</tbody>
</table>
that the auricles, not the staminode lobes, offer a liquid reward to pollinators in three Corunastylis species. It is therefore suggested that, within the genus Corunastylis, there may be more than one secretory site in the flowers. Different feeding locations may, in turn, facilitate divergent foraging behaviors of pollinators leading to either head or thorax deposition of pollinia (Kuiter 2018). Garnet (1940) observed chloropids feeding on labellum nectar in C. archeri and C. nigricans. He concluded that, while the self-inverted fly foraged for nectar at the base of the labellum callus, the weight of the insect caused the jointed labellum to collapse onto the column. This collision transferred the viscidium to the dorsum of the fly’s thorax, releasing the stipe and its attached pollinia. There is now a second option based on our location of a second reward site. The labellum may also tilt into the receptive column while the insect attempts to forage on auricle secretions.

**Comparative presentation of column appendages in three Corunastylis species versus other orchids**

Burns-Balogh & Bernhardt (1985) proposed that the column appendages and/or staminodia of some lineages in Orchidaceae were often functional and should not always be dismissed as vestigial organs. The most obvious example within Diurideae is found in large-flowered species in the genus Thelymitra. Each pair of staminodes in the same flower is usually connate, forming an ornamented hood attracting foragers that ultimately contact the viscidium and receptive stigma lobes (see Edens-Meier & Bernhardt 2014). Excluding the wet stigma lobes, the secretion of fluids by column organs remains rare in Orchidaceae. The most often cited example is in the epidendroid genus, Coryanthes (Stanhopeinae). A pair of glands, known as pleuridia, flank the base of the column. Pleuridia may actually represent the extended and much modified bases of the column appendages, adnate to the lower style (Gerlach 2011). These glands secrete a watery slime into the bucket-like lamina of the labellum. The only known pollinators are male euglossine bees that fall into the bucket as part of the pollinarium dispersal process. The accumulated liquid lacks edible rewards and appears to slow bee escape (Roubik 2014).

The auricles of all Corunastylis species examined contained clusters of raphides. In orchids, raphides produce mucilage (Smith 1923). Raphide clusters in the auricles of C. filiformis, C. fimbriata and C. ruppii might produce dilute mucilage, contributing to a diluted or viscous (e.g. C. ruppii) reward. It was not possible to observe auricle secretions in the remaining three species, and Garnet (1940) showed previously that the same species produce nectar at the bases of their labellum calluses. It was noted that the auricle of C. archeri is so reduced in size, compared to the auricles of C. filiformis, C. fimbriata and C. ruppii, that it could be interpreted as a vestigial organ. However, as all auricles of all species studied contain raphide clusters, it could be that these cells no longer contribute to the pollination mechanism in some Corunastylis species. Perhaps raphide clusters in auricles of some Corunastylis species have additional functions, as they do in the floral organs of some Neotropical orchids also pollinated by micro-dipterans. For example, they could play a role in the synthesis of trace nutrients or scents as suggested for Trichosalpinx species (Bogarin et al. 2018). Their presence could also present a refractile visual cue that helps attract and orientate pollinators, as proposed for Stelis aff. purpurescens A. Rich. & Galeotti (Chase & Peacock 1987). Specifically, raphides in Corunastylis species could make the auricles appear shinier and more attractive to incoming chloropids as they are present in such a thin matrix of tissue.

It was also noted that secretory column appendages remain unreported in the allied genus Prasophyllum (as Chiloterus, Mecopodum and Prasophyllum s.s in Jones 2006). These taxa are more likely to be pollinated by larger bees, wasps and syrphid flies (Weston et al. 2014). Floral structures in this genus also lack the trembling labellum (Jones 2006). Nicholls (1969) completed descriptions and detailed iconographies of many Prasophyllum species. Examination of his plates and descriptions of 44 species show glabrous to sub-glabrous staminodia and much-reduced auricle lobes. A few species show auricles with a wrinkled to lumpy epidermis (e.g. Prasophyllum flavum R.Br.). Nicholls also wrote that the column appendages of many of these species were “inconspicuously bilobed” or that the column appendage was “short lobed” at its base.
Specialised floral scents

The molecule identified as 8-heptadecene appeared to dominate the scent of Corunastylis filiformis (70.1–73.06%) but was found in only 3 out of 4 inflorescences of C. ruppii (4.89–24.17%). The presence of tridecane (11.90–30.32%) in C. ruppii is of particular interest as it may attract Conioscinella species (Chloropidae) to flowers in the genus Ceropedia (Apocynaceae) according to Heiduk et al. (2017). Kaiser (2011) detected this molecule in the floral scents of more than 35 species in seven families of monocots, eudicots and as a trace in the eumagnoliid, Magnolia delavayi Franch. This included 19 orchid species representing three subfamilies (Cypripedioideae, Orchidoideae, Epidendroideae), but in far lower proportions (0.03–3.0%) compared to C. ruppii. The loss of scents in our collections of C. fimбриata due to long-term storage was unfortunate as six additional compounds were present but too low in volume to identify (unpublished). The lemony odor of its fresh flowers would suggest citronellol or citronellol (R. Raguso, pers. comm.). The presence of nepetalactone in C. fimбриata is novel as it was first isolated in stems and leaves of catnip (Nepeta cataria L. (Lamiaceae)). This molecule does attract cats (McElvain et al. 1941) but commercial concentrations are also reputed to repel some mosquitos (Aedes spp. (Culicidae); see Kingsley 2001).

Interpreting the suite of floral traits

Based on the evidence presented, there are now two possible interpretations of floral presentation in Corunastylis filiformis, C. fimбриata and C. ruppii. These two interpretations may, in fact, overlap. First, the high proportion of tridecane in C. ruppii allies with past work showing that female chloropids are attracted to wounded heteropterans (Zhang & Aldrich 2004). Dodecen-1-ol and 8-heptadecene, derived from C. ruppii, are similar in structure to the long chain hydrocarbons and carboxylic acids (see Bogarin et al. 2018) identified in Trichosalpinx species, pollinated by blood drinking females in the Ceratopogidae. In this case, the viscous or sticky exudate of the auricles of C. ruppii may simulate haemolymph and would help explain the comparative absence of labellum movement unless it is probed. Bower et al. (2015) were the first to propose this model in Corunastylis littoralis without a scent analysis.

Second, in Australia, chloropids are commonly referred to as eye gnats or eye flies as the winged adults are known to feed on lachrymal secretions as they move from orifice to face (Matheson 1950) and may be trapped using a range of scents (Rogoff et al. 1973; Heiduck et al. 2017). It is suggested here that the floral secretions, combined with the hairy, darkly pigmented labella and staminodes of some Corunastylis species, may mimic the respective tears and eyelids of mammals. Borba & Semir (1998) suggested that wind was necessary to effect pollinarium transfer in Bulbophyllum ipanemense Hoehne as the weight of the tiny fly was insufficient to tilt the labellum towards the column. While the authors agree that the hinged, cantilevered labellum in C. filiformis and C. fimбриata may also operate under a wind-facilitated system, a second function should also be considered for the same modified petals. Specifically, a fluttering and hairy labellum is mimetic and helps to attract those micro-dipterans that are the primary dispersers of pollinaria. Of course, this new interpretation is ultimately dependent on future chemical analyses of auricle secretions. If signature salts and amino acids are identified in these secretions, it is reasonable to suggest that these flowers are winking at their prospective pollinators!

Acknowledgments

This research was funded by the Australian Orchid Foundation (project 303.15). We are grateful to the Royal Botanic Gardens and Domain Trust, Sydney, New South Wales, for their ongoing logistical support, lab access and budget management under Dr. Peter Weston as Senior Principal Research Scientist and now Honorary Research Associate. We thank Mr. Chris Gibson for access to his property along the Bloodwood Road. We also wish to thank Ms. Zhen Yu of the Kunming Institute of Botany, Chinese Academy of Sciences, for her help with the GC-MS analyses of scents. We thank Dr. D. Bickel, of the Australian Museum (Sydney), for his expertise in identifying the fly in Figure 2. We are grateful to Rudie Kuiter for sharing his observation of flowers of Corunastylis spp. and taking us to his Crib Point and Langwarrin study populations, and to Dr. Robert Raguso (Cornell University) for discussing origin and functions of volatile particles.

Second, in Australia, chloropids are commonly referred to as eye gnats or eye flies as the winged adults are known to feed on lachrymal secretions as they move from orifice to face (Matheson 1950) and may be trapped using a range of scents (Rogoff et al. 1973; Heiduck et al. 2017). It is suggested here that the floral secretions, combined with the hairy, darkly pigmented labella and staminodes of some Corunastylis species, may mimic the respective tears and eyelids of mammals. Borba & Semir (1998) suggested that wind was necessary to effect pollinarium transfer in Bulbophyllum ipanemense Hoehne as the weight of the tiny fly was insufficient to tilt the labellum towards the column. While the authors agree that the hinged, cantilevered labellum in C. filiformis and C. fimбриata may also operate under a wind-facilitated system, a second function should also be considered for the same modified petals. Specifically, a fluttering and hairy labellum is mimetic and helps to attract those micro-dipterans that are the primary dispersers of pollinaria. Of course, this new interpretation is ultimately dependent on future chemical analyses of auricle secretions. If signature salts and amino acids are identified in these secretions, it is reasonable to suggest that these flowers are winking at their prospective pollinators!

Acknowledgments

This research was funded by the Australian Orchid Foundation (project 303.15). We are grateful to the Royal Botanic Gardens and Domain Trust, Sydney, New South Wales, for their ongoing logistical support, lab access and budget management under Dr. Peter Weston as Senior Principal Research Scientist and now Honorary Research Associate. We thank Mr. Chris Gibson for access to his property along the Bloodwood Road. We also wish to thank Ms. Zhen Yu of the Kunming Institute of Botany, Chinese Academy of Sciences, for her help with the GC-MS analyses of scents. We thank Dr. D. Bickel, of the Australian Museum (Sydney), for his expertise in identifying the fly in Figure 2. We are grateful to Rudie Kuiter for sharing his observation of flowers of Corunastylis spp. and taking us to his Crib Point and Langwarrin study populations, and to Dr. Robert Raguso (Cornell University) for discussing origin and functions of volatile particles.

Second, in Australia, chloropids are commonly referred to as eye gnats or eye flies as the winged adults are known to feed on lachrymal secretions as they move from orifice to face (Matheson 1950) and may be trapped using a range of scents (Rogoff et al. 1973; Heiduck et al. 2017). It is suggested here that the floral secretions, combined with the hairy, darkly pigmented labella and staminodes of some Corunastylis species, may mimic the respective tears and eyelids of mammals. Borba & Semir (1998) suggested that wind was necessary to effect pollinarium transfer in Bulbophyllum ipanemense Hoehne as the weight of the tiny fly was insufficient to tilt the labellum towards the column. While the authors agree that the hinged, cantilevered labellum in C. filiformis and C. fimбриata may also operate under a wind-facilitated system, a second function should also be considered for the same modified petals. Specifically, a fluttering and hairy labellum is mimetic and helps to attract those micro-dipterans that are the primary dispersers of pollinaria. Of course, this new interpretation is ultimately dependent on future chemical analyses of auricle secretions. If signature salts and amino acids are identified in these secretions, it is reasonable to suggest that these flowers are winking at their prospective pollinators!
References
Kuiter, R. (2018). Pollination of Victoria’s leek orchids & their allies. Aquatic Photographics, Seaford, Victoria, Australia


