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ORIGINAL ARTICLE

Pollination biology of *Luisia curtisii* (Orchidaceae): indications of a deceptive system operated by beetles

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Abstract A population of *Luisia curtisii* (Orchidaceae: Aeridinae) in northern Thailand was studied with regard to pollination biology. Although a high level of self-compatibility was demonstrated experimentally, the very low natural fruit set (1.4–1.9 %) clearly indicated that the species depends on external agents for pollination. Our observations suggest that *L. curtisii* is pollinated by beetles, as *Lema unicolor* (Chrysomelidae) and *Clinteria ducalis* (Scarabaeidae) were the only flower visitors observed to carry pollinaria of this species. The hypothesis of specialised cantharophily is further supported by 2-methylbutyric acid and caproic acid being striking components of the floral scent. Judging from the lack of nectar and the behaviour of visiting beetles, the pollination system seems to rely on food or brood site deception. Retention of the anther on the pollinarium for some time after pollinarium removal probably reduces the frequency of insect-mediated autogamy and geitonogamy in *Luisia curtisii*—a possibility that was supported by comparative data on (1) the anther retention time and inflorescence visitation time of *Lema unicolor* and (2) stigma and anther length in the orchid. Existing reports of specialised beetle pollination in orchids are reviewed, and we conclude that there is accumulating evidence that specialised cantharophily is more common in the Orchidaceae than previously assumed.

Keywords Allogamy · Anther retention · Cantharophily · Floral scent · Fruit set · Outcrossing

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Introduction

Ever since Darwin (1862) published his epoch-making book on the various contrivances by which orchids are pollinated by insects, a rapidly increasing number of orchid species have become objects of a continuously developing and widening field of anthecological research (Nilsson 1992; Micheneau et al. 2009). Nevertheless, the pollination biology—one of the key factors to understanding the diversification of orchids (Tremblay et al. 2005; Vereecken et al. 2010)—still remains completely unknown for the vast majority of tropical orchid species, especially those in the Old World (van der Cingel 2001). The SE Asian to western Pacific genus *Luisia* (Seidenfaden 1971) of the epidendroid subtribe Aeridinae is one of many genera from which not a single possible pollination event has ever been recorded.

To help remedy this lack of knowledge, we performed a field study of pollination in one of the ca. 30 known *Luisia* species, i.e. *L. curtisii* Seidenf., which occurs in evergreen forests in Vietnam, Thailand, Peninsular Malaysia and

Borneo (Seidenfaden 1988). The main purpose of our study was to reveal the diversity and identity of insects carrying pollinaria from *L. curtisii* and to evaluate the success of the pollination system by assessing the level of natural fruit set in the study population. Endress (1994) suggested that pseudocopulation stimulated by sexual deceit of insects might play a role for pollination of the "peculiar" flowers of *Luisia*. Therefore, we also wanted to check whether *L. curtisii* offers a floral reward or whether its flowers deceive the pollinators. Finally, we wanted to identify adaptations (if any) that reduce the probability of self-pollination in our study species. Many such adaptations have been reported from orchids, including: self-incompatibility, functional dioecy, protandry, deception, flowers in which the pollinator is forced to pass the stigma prior to the pollinarium, change of shape, orientation or size of the pollinarium following its removal from the anther and retention of the anther on the pollinarium for some time after pollinarium removal (e.g. Darwin 1862; van der Pijl and Dodson 1966; Catling and Catling 1991a, b; Borba and Semir 1999; Tremblay et al. 2005; Jersáková et al. 2006; Peter and Johnson 2006b). Preliminary observations on *L. curtisii* suggested that anther retention might well serve to prevent self-pollination in this species (Pedersen et al., unpublished data).

As we soon realised that the flowers of *L. curtisii* are almost exclusively visited by beetles, we also took the opportunity to review the previously published information on cantharophilous orchids in order to provide an up-to-date survey of specialised beetle pollination in this family.

Materials and methods

Study species and study population

Luisia curtisii (Fig. 1) is a monopodial epiphyte/lithophyte with a branched, creeping stem. The spreading to erect part of a flowering shoot (Fig. 1a) is 9–60 cm long and carries 1–7 alternate terete leaves. One or two (very rarely three) short and dense racemes emerge through the leaf sheaths more or less opposite the laminas; they carry (1–)2–5(–10) dull yellowish-white flowers suffused with violet, but only 1–2(–3) flowers in a raceme are open at the same time (Fig. 1b). Individual flowers usually last for 1–2 weeks (but if they are pollinated they wither in 1–4 days). The dorsal sepal is parallel to the column, whereas the lateral sepals are parallel to the labellum and describe an acute angle to each other; they are all strongly boat-shaped. The lateral petals are porrect over the column, linear and distally incurved. The somewhat reflexed labellum is fleshy and differentiated into hypochile and epichile. The subrectangular hypochile is smooth and with low erect side lobes in its proximal half; it is 4.2–4.6 mm long along the midline, 3.9–4.0 mm wide at the junction with the epichile, and the distance between the apices of the two side lobes is 2.5–3.0 mm. The slightly vaulted epichile is broadly cordate, irregularly longitudinally furrowed and rounded to retuse; it measures 4.1–5.2 mm along the midline, and its maximum width is 6.5–8.8 mm. The straight to slightly incurved column is ca. 4.5 mm long and more or less perpendicular to the labellum. The fertile stigma is transversely elliptic and strongly concave, the anther (Fig. 1c) versatile. The pollinarium (Fig. 1d) is ca. 2.3 mm long and consists of 2 porate pollinia, 2 tiny (but extremely elastic) caudicles, an oblong tegula and a transversely angular-elliptic viscidium.

Our study was conducted at Lan Hin Taek in Phu Hin Rong Kla National Park (northern Thailand, province of Phitsanulok) where a large population of *L. curtisii* grows lithophytically on exposed sandstone boulders in hill evergreen forest at ca. 1,200 m altitude.

Breeding system

Twelve flowers on eight shoots were experimentally self-pollinated on 18–20 March 2011 to test for self-compatibility. Three flowers on three shoots of other individuals were cross-pollinated for comparison. Fruit set was assessed by eye, and on 1 November the seed quality was evaluated under light microscope by counting the numbers of seeds with and without a well-developed embryo, respectively. For each fruit, three random samples were assessed, each comprising 1,035–1,268 seeds. A Student's *t* test was performed (in the programme SigmaStat network 1.01) to test for differences between the proportion of embryo-containing seeds in fruits resulting from self-pollinations and crosses, respectively.

Nectar and floral scent

Flowers were examined for nectar in situ—partly by eye and hand lens, partly by sticking small glucose test strips (Bayer Clinistix) into crevices and furrows of the labellum. In a greenhouse of the Stadtgärtnerei Zürich (Switzerland), floral scent of entire flowers of a cultivated plant (collection no. A. Kocyan AK586) was collected by headspace adsorption: flowers were placed into a suitable glass chamber and the air containing the scent was pumped through an adsorption trap (3 mg of Poropak Super Q from Sigma-Aldrich, http://www.sigmaaldrich.com) driven by an SKC 222-4 (SKC Inc., http://www.skcinc.com) air sampler. Samples were eluted with 50 µl of a mixture of highly pure hexane and acetone (10:1); 1 µl of the eluate was injected into a gas chromatograph (Carlo Erba Fractovap 4160, Thermo Scientific) or a GC-MS (Thermo

Fig. 1 Morphology of *Luisia curtisii*. **a** Habit; **b** inflorescence; **c** ventral view of a pollinarium experimentally removed from a flower; the anther is still retained, but the ventral flaps have started to open; **d** same as **c**, but the anther has disappeared, exposing the pollinia; **e** capsules. Photos by H. Æ. Pedersen

Finnigan Voyager Mass Spectrometer). DB-Wax columns (J & W Scientific, Folsom, CA, USA) of 30 m × 0.32 mm with a film thickness of 0.25 μm were used for analyses. Subsequently, compounds were comparatively identified with mass spectra and retention times of existing reference samples (for methodological details, see Kaiser and Tollsten 1995; Johnson et al. 2005). Scent analysis was performed by Roman Kaiser (Givaudan Natural Scents, Dübendorf, Switzerland).

Flower visitors

Flower visitors were observed and their behaviour described, and the position and number of pollinaria (if any) were noted for each individual visitor. A few individuals of each visiting insect species were collected for later identification by Jan Bezděk and Alexey Solodovnikov; insect vouchers are deposited at DEFACU, JBCB and ZMUC. Observations were made on 28 March 2005 (8:30–12:30, local

time, two observers), 20 March 2010 (13:00–17:00, three observers), 21 March 2010 (6:30–16:00, three observers), 14 March 2011 (12:00–15:15, three observers), 15 March 2011 (6:45–9:30, 14:00–17:30, three observers) and 18 March 2011 (10:00–12:15, two observers; 12:45–14:00, three observers). Thus, a total of 85.25 man h was spent observing and collecting flower visitors during the peak flowering season in 2005 and 2010 and during the early phase of flowering in 2011. During our field work in 2005 and 2010, the weather was consistently sunny with daytime temperatures ranging from 38–42 °C. In 2011, on the other hand, the weather was cloudy with intermittent showers and daytime temperatures ranging from 15 to 25 °C.

In order to check for nocturnal visitors attracted by floral scent, two traps (diameter of entrance: 10 cm), each containing 15 *L. curtisii* flowers as bait, were placed in the study population at 19:00 on 20 March 2010 and checked at 6:30 the following morning.

Anther retention

While preparing the study, we noticed that the anther was retained on the pollinarium when the latter was experimentally removed from the flower (Fig. 1c). As this suggested an adaptation preventing self-pollination and geitonogamy, we compared the lengths of the anther, stigma and pollinia by measuring these structures in 31 *L. curtisii* flowers after 2 days storage in 70 % ethanol (the idea being to check if the anther was sufficiently large to act as a shield between the pollinia and stigma). The widths of the same organs, on the other hand, were not measured, because it was evident that the anther was never wider than the stigma. Using the programme SigmaStat network 1.01, a Kruskal-Wallis one-way ANOVA (analysis of variance) on ranks was performed to test whether the anther, pollinia and stigma differed in length. The non-parametric test was chosen because the data set failed a normality test. Following a positive outcome of the ANOVA, a Student-Newman-Keuls test was run to reveal which organ(s) differed from the others.

During our field work in 2011, we attempted to score the duration of each observed inflorescence visit (usually identical with "flower visit" because of the sequentially opening flowers) with the intention to compare the relation between visitation time and the duration of anther retention. The duration of anther retention was assessed experimentally. Using a wooden tooth pick, pollinaria were removed from 50 flowers in situ; in each case the pollinarium was immediately placed in a position where it was exposed to a light air current (ca. 0.4 m s^{-1}), and the time it took for the anther to shrivel and fall off was recorded. Using SigmaStat, a Mann-Whitney rank sum test was performed to test whether anther retention time and the duration of active inflorescence visits by insects were different. The non-parametric test was chosen because the data set failed a normality test.

Natural fruit set

At the time of dehiscence, the capsules of *L. curtisii* are strongly lignified (Fig. 1e), and they persist on the old inflorescence throughout the flowering season of the following year. On 22 March 2010 we could therefore assess the natural fruit set of 2009 by counting the number of capsules on 186 old inflorescences belonging to 158 individual shoots, whereas the relative fruit set was calculated as the number of capsules divided by the total number of flower nodes on the same inflorescences. A corresponding estimate of the natural fruit set of 2010 was obtained from 231 old inflorescences on 198 individual shoots examined on 18 March 2011.

To get an instantaneous impression of the magnitude of pollinaria loss, we examined 908 *L. curtisii* flowers in situ on 18 March 2011, and for each of them we noted whether the pollinarium had been removed and whether a pollinarium had been deposited on the stigma.

Results

Breeding system

Fruit set in the experimentally self-pollinated and cross-pollinated flowers was 100 %. More than 7 months after the hand pollinations had been conducted (during which period five of the capsules resulting from self-pollination were shed), the seeds were still not completely mature. However, they had almost reached their final size, and it was easy to distinguish between embryo-containing and empty seeds. All capsules were packed with seeds of generally high quality, but the seed quality was significantly lower in capsules resulting from self-pollinations than in those resulting from crosses ($P < 0.0001$). Thus, the proportion of embryo-containing seeds in capsules produced by self-pollinated flowers ranged from 90.1 to 91.5 % (mean 90.9 %), whereas it ranged from 96.7 to 97.4 % (mean 97.2 %) in capsules produced by cross-pollinated flowers.

Nectar and floral scent

No floral nectar could be observed or detected by glucose test strips. GCMS analysis of the floral scent collected from plants cultivated in Zürich identified 14 constituents (Table 1), of which 2-methylbutyric acid (3.3 %) and caproic acid (0.2 %; both fatty acids with a somewhat

Table 1 List of relative amounts of the floral scent compounds detected in *Luisia curtisii* flowers. Compounds are sorted according to the elution performance on a DB-Wax capillary column

Limonene	1.20 %
6-Methyl-5-hepten-2-One	0.50 %
Nonanal	0.50 %
Decanal	0.30 %
β-Cubebene	0.20 %
Caryophyllene	69.00 %
Humulene	8.40 %
2-Methylbutyric acid	3.30 %
Germacrene A	0.50 %
δ-Cadinene	0.30 %
Caproic acid	0.20 %
Caryophyllene epoxide	4.10 %
Humulene epoxide II	0.30 %
para-Cresol	0.03 %
Total	88.83 %

The total represents the amount of volatile odour substances; the remaining difference can be attributed to contaminants from the environment

unpleasant smell) are odour-wise the most striking components; caryophyllene (69 %) has the highest relative proportion, but it has a high recognition threshold and contributes little to the floral bouquet for the human nose; humulene (8.4 %) and caryophyllene epoxide (4.1 %) are further constitutents with larger proportions.

Flower visits

Some individuals of *L. curtisii* were heavily infested by aphids on the upper part of the stem and sometimes on the inflorescences. Small ants occasionally attended the aphids, but even the ants were much too small to remove or transport pollinaria. No insects were caught in the baited nocturnal traps, and apart from aphids and ants, only two beetle species were observed as flower visitors on *L. curtisii*.

On 19 occasions we observed *Lema unicolor* Clark (Chrysomelidae) visit flowers of *Luisia curtisii* (Fig. 2a–c). In five cases (classified as "inactive visits", only observed on cool and humid days) the beetles were completely passive and apparently rested on the flower, usually on the dorsal side of a sepal. In the remaining 14 cases (classified as "active visits") the beetles entered the flower and consistently probed the furrows and crevices of the labellum and its junction with the column (Fig. 2c). Only once did we see *Lema unicolor* damage a flower by gnawing one of the petals (despite its close relative *L. pectoralis* Baly being known as a florivorous pest in orchid nurseries); in general we noticed only little floral damage caused by herbivory in *Luisia curtisii*. The beetles entered the flowers in various ways and did not consistently assume a position that would facilitate pollination (Fig. 2a, b). Nevertheless, one *Lema unicolor* individual was carrying a *Luisia curtisii* pollinarium (with no anther retained) on the hind edge of one of its fore wings (elytra).

We also observed two individuals of *Clinteria ducalis* White (Scarabaeidae) as active flower visitors on *L. curtisii*, and one of them (Fig. 2d) was carrying an *L. curtisii* pollinarium on its head. In addition, one individual of *C. ducalis*, carrying at least two *L. curtisii* pollinaria on its head, was observed in a *Syzygium* flower (Myrtaceae). In no case was the anther retained on pollinaria observed on the beetles. The two individuals of *C. ducalis* visiting *L. curtisii* flew directly to the flowers where they probed the furrows of the labella.

Anther retention

The anther, stigma and pollinia, respectively, measured 2.1 ± 0.1, 1.8 ± 0.2 and 1.4 ± 0.1 mm (indicated as mean ± SD); see also Fig. 3. The three organs were of different length ($P < 0.001$); thus, the anther was longer than the stigma ($P < 0.05$), which in turn was longer than the pollinia ($P < 0.05$).

On seven occasions we succeeded in scoring the duration of an individual, active inflorescence visit by *Lema unicolor* (although none of these beetles carried pollinia). The duration of individual inflorescence visits was 126 ± 99 s (indicated as mean ± SD); see also Fig. 4a. Inactive visits could last up to 40 min (possibly even longer). However, we did not consider it relevant to include inactive visits when comparing the duration of inflorescence visits and anther retention, as the behaviour of inactive, resting beetles would never lead to pollen removal or deposition anyway. Upon experimental removal of a pollinarium (retaining the anther), the two ventral flaps of the anther gradually opened (Fig. 1c) until the anther no longer grasped the pollinia, after which it fell off (Fig. 1d). The duration of anther retention was 289 ± 242 s (indicated as mean ± SD); see also Fig. 4b. Anther retention time was found to be longer than active flower visits of *L. unicolor* ($P < 0.02$).

Natural fruit set

Natural fruit set in 2009 was 1.9 % ($N = 697$). Out of the 158 shoots checked, 131 carried one inflorescence (with 0, 1 or in one case 2 capsules), 26 shoots carried two inflorescences (with 0 or 1 fruit), and one shoot carried three fruitless inflorescences. Natural fruit set in 2010 was 1.4 % ($N = 768$). Out of the 198 shoots checked, 165 carried one inflorescence, whereas 33 carried two inflorescences; in no case did an inflorescence carry more than one capsule.

Fig. 2 Flower visits and pollination of *Luisia curtisii*. **a**, **b** *Lema unicolor* assuming different positions inside the flower; **c** *Lema unicolor* probing a furrow in the labellum; **d** *Clinteria ducalis* with a *Luisia curtisii* pollinarium deposited on its head; **e** pollinated flower that has lost its own pollinarium (and anther). Photos by H. Æ. Pedersen

Our examination of 908 *L. curtisii* flowers revealed that 53 of them (5.8 %) had lost their own pollinarium, whereas only three (0.3 %) had received a pollinarium on the stigma. In one flower, both actions had taken place (Fig. 2e). The combination of 53 pollinaria removed and only 3 deposited indicates that ca. 94 % of the removed pollinaria were lost.

Discussion

Pollinators and pollination system

The circumstance that experimental self-pollinations resulted in 100 % fruit set and reasonably high seed quality (90.1–91.5 % embryo-containing seeds compared to 96.7–97.4 % in capsules resulting from crosses) indicates that *L. curtisii* has a high level of self-compatibility, also when compared with the magnitude for most of the orchid species reviewed by Tremblay et al. (2005) and Jersáková et al. (2006). However, it cannot be ruled out that the shedding of five unripe capsules produced by self-pollination was a result of severe inbreeding depression rather than an effect of resource limitation. Under all circumstances, the very low natural fruit set (1.4–1.9 %) strongly suggests that the flowers are not spontaneously autogamous, but depend on external agents for pollination.

Our observations of the chrysomelid *Lema unicolor* (Fig. 2a–c) and the scarabaeid *Clinteria ducalis* (Fig. 2d) carrying pollinaria of *Luisia curtisii* suggest that this

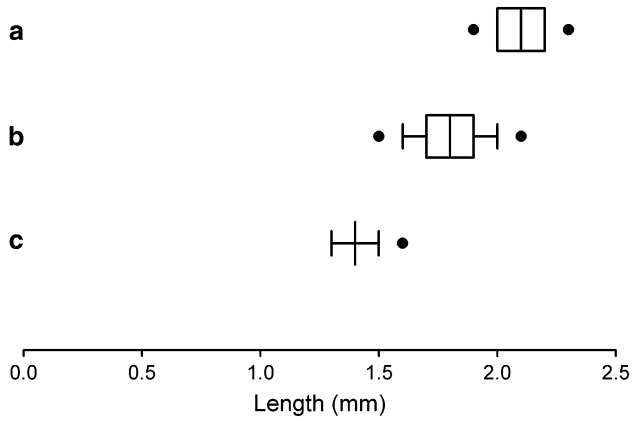

Fig. 3 Box plot showing the lengths of **a** the anther, **b** the stigma and **c** the pollinia of *Luisia curtisii*. *Length* of *each box* represents the interquartile range (median value as *vertical line*); *horizontal lines* represent the minimum and maximum values, excluding outliers, which are indicated by *dots*

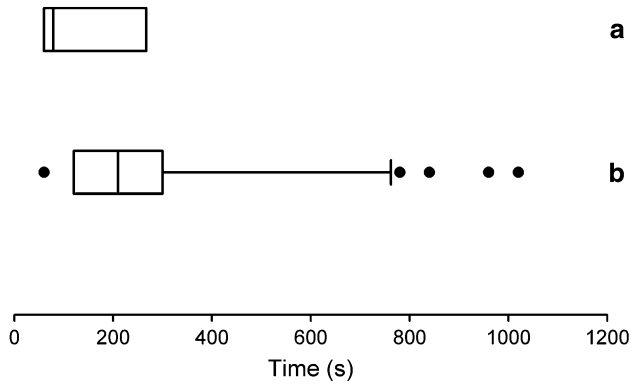

Fig. 4 *Box plot* showing **a** the duration of active inflorescence visits by *Lema unicolor* and **b** the variation in anther retention time in *Luisia curtisii*. *Length of each box* represents the interquartile range (median value as *vertical line*); *horizontal lines* represent the minimum and maximum values, excluding outliers, which are indicated by *dots*

species is beetle-pollinated. Admittedly, the low share of beetles carrying pollinia might indicate that, although they were the only observed flower visitors of adequate size, they are not the primary pollinators. However, it does not seem likely that we completely missed the primary pollinators during 85.25 h of observation. Moreover, dull-coloured flowers with an unpleasant scent and with an easily accessible, flat and fleshy labellum devoid of nectar guides (Fig. 1b) are indeed features that could (by inference) be expected in a beetle-pollinated orchid (cf. Faegri and van der Pijl 1966; Gottsberger 1999). In particular, caproic acid is known to be a powerful attractant to certain beetle species (e.g. Poprawski and Yule 1992). However, to test the direct electrophysiological activity of the various odour components, a study using gas chromatography coupled with electroantennographic detection (GC/EAD) that exposes insect antennae to the individual compounds of the floral bouquet should be conducted (Schiestl and Marion-Poll 2001). Finally, the fact that we consistently observed each pollinarium-bearing beetle right from its arrival to a *Luisia curtisii* inflorescence (combined with the fact that no pollinarium observed on the beetles still retained the anther) shows that the pollinaria must have been brought there from other *L. curtisii* inflorescences. Observation of actual pollen deposition on a stigma could have finally confirmed that beetles pollinate *L. curtisii*. However, such an observation would have been extremely lucky in a population with 1.4–1.9 % natural fruit set and 94 % of the removed pollinaria being lost—see also Alexandersson and Ågren's (1996) corresponding (lack of) observations in the likewise rarely pollinated *Calypso bulbosa* (L.) Oakes.

The very direct approach of the *C. ducalis* individuals (more direct than that of *Lema unicolor*) suggests that not only olfactory, but also visual cues were important for attracting them. The circumstance that pollinaria were more frequently observed on *C. ducalis*, in which species they were consistently deposited on the head (Fig. 2d), indicates that this species may be a more efficient pollinator than *L. unicolor*, which, on the other hand, visited *Luisia curtisii* flowers much more frequently.

Since no reward was offered by the flowers, they must be pollinated by deceit and, indeed, the very low natural fruit set (1.4–1.9 %) matches the level reported for many other deceit-pollinated orchid species (e.g. Neiland and Wilcock 1998; Tremblay et al. 2005; Scopece et al. 2010). The lack of pseudocopulation behaviour of the beetles and their probing of furrows and crevices of the labellum (Fig. 2c) suggest that we are dealing with food or brood site deception, rather than sexual deception as predicted by Endress (1994). In this context, it should be noted that several other species of *Luisia* produce copious nectar that is freely accessible from the basal part of the labellum (Kocyan et al., in preparation).

Anther retention

The observed gradual opening of the ventral flaps of the anther upon pollinarium removal from the flower (Fig. 1c) suggests desiccation of anther cells to be responsible for the shedding of the anther and for the timing of this process. Evidence for water loss as the primary mechanism behind dropping of the anther was given by Peter and Johnson (2006a) for *Eulophia foliosa* (Lindl.) Bolus, as they demonstrated the process of anther release to occur more rapidly when the water vapour gradient from the anther cell to the atmosphere was large during conditions of high vapour pressure deficit.

In previous studies of other orchid species, the duration of anther retention has been found to be consistently longer

than average flower (or inflorescence) visitation time, clearly demonstrating the importance of anther retention for reducing the probability of insect-mediated self-pollination or geitonogamy in those species (Singer and Cocucci 1999; Borba and Semir 2001; Peter and Johnson 2006a, b). In *Luisia curtisii*, we found a similar difference between anther retention time and the duration of active inflorescence visits by *Lema unicolor* (Fig. 4). Unfortunately, we did not obtain data on the visitation time of the apparently more efficient pollinator *C. ducalis*. However, the circumstance that the anther is significantly longer than the stigma in *Luisia curtisii* (Fig. 3)—thus temporarily preventing its pollinia from being deposited on a stigma—adds further support to the hypothesis that also in *L. curtisii*, anther retention is an adaptation that increases the relative frequency of outcrossing. In a future study it would be interesting to test the assumed low level of autogamy by quantifying the actual occurrence of self-pollination across the population (using, as reference, the significantly different shares of embryo-containing seeds from experimental self-pollinations and crosses, respectively). A similar approach was used by Peter and Johnson (2009) in their study of *Acrolophia cochlearis* Schltr. & Bolus.

Beetle pollination in orchids

If "specialised beetle pollination" is defined as pollination performed exclusively by one or more species of beetles, five cases have been reported from the southern hemisphere. In Argentina, Singer and Cocucci (1997) observed *Eulophia ruwenzoriensis* Rendle (sub syn. *Pteroglossaspis ruwenzoriensis* (Rendle) Rolfe) to be pollinated by the scarabaeid beetle *Euphoria lurida* (Fabricius). In South Africa, Steiner (1998) observed pollination by the scarabaeid beetles *Heterochelus podagricus* Fabricius and *Lepithrix hilaris* Peringuey in *Ceratandra grandiflora* Lindl.; Peter and Johnson (2006b) reported *Eulophia parviflora* (Lindl.) A.V. Hall to be pollinated by scarabaeid beetles of the subfamily Cetoniinae; and Peter and Johnson (2006a) convincingly demonstrated *E. foliosa* to be pollinated by the elaterid beetle *Cardiophorus obliquemaculatus* Schwarz. Finally, in Queensland (Australia), *Peristeranthus hillii* (F. Muell.) T.E. Hunt is visited and possibly pollinated by the lycid beetle *Metriorrhynchos rufipennis* Fabricius; at least, this species has been observed to carry *P. hillii* pollinaria on its antennae (Wallace 1980; Forster 1988).

Beside the likely case of *Luisia curtisii* presented in this article, there are three documented cases of specialised beetle pollination in northern hemispere orchids. First, Pradhan (1983) reported the Himalayan *Vanda cristata* Wall. ex Lindl. to be eagerly visited and pollinated by an unidentified species of beetle; his photos show that we are dealing with a chafer (Scarabaeidae). Second, the eastern Mediterranean *Ophrys fusca* Link subsp. *blitopertha* (Paulus & Gack) N. Faurh. & H.A. Pedersen (syn.: *O. blitopertha* Paulus & Gack, *O. urteae* Paulus) is pollinated by the chafers *Blitopertha lineolata* Fischer von Waldheim (Paulus and Gack 1990, 1992; Paulus 1998) and *B. nigripennis* Reitter (Paulus 2009). Third, Jin et al. (2005) observed the southern Chinese *Holcoglossum rupestre* (Hand.-Mazz.) Garay to be pollinated by *Hybovalgus bioculatus* Kolbe (Scarabaeidae: Cetoniinae). It should be added that a frequently cited observation of beetle pollination in *Dendrochilum longifolium* Rchb.f. (Ridley 1896: 230) in all probability represents a casual event in a much less specialised pollination system operated by a range of facultatively anthophilous insects (Pedersen 1995).

According to van der Pijl and Dodson (1966), the cases of beetle pollination in orchids "…are obviously scattered in the lower orchids and there appears to be no trend toward adaptation to beetles as pollinators". However, there is accumulating evidence that specialised beetle pollination is more common in orchids (especially in the tropics) than previously assumed (see also Bernhardt 2000; van der Cingel 2001), and so far it has been reported from both the orchidoid tribe Orchideae (subtribes Disinae, Orchidinae) and the epidendroid tribes Cymbidieae (subtribe Eulophiinae) and Vandeae (subtribe Aeridinae). By now, it also seems that cantharophilous adaptations in orchids do exist. Thus, orchid species that depend on specialised pollination by beetles mostly have open flowers with flat to shallow (rarely spurred) labella, and their flowers either emit fruity to yeasty scents, faint honey-like scents, butyric acid or caproic acid (this study; Wallace 1980; Singer and Cocucci 1997; Peter and Johnson 2006a) or scents that are assumed to mimic sexual pheromones of the specific pollinator species (Paulus and Gack 1990; Paulus 1998; Knudsen et al. 2006). Furthermore, specialised beetle pollination in orchids has turned out to involve a diversity of types, including systems with nectar reward (Wallace 1980; Forster 1988; Singer and Cocucci 1997; Jin et al. 2005) and deceptive systems involving pollination through foraging behaviour of deceived beetles (Pradhan 1983; Peter and Johnson 2006a), rendezvous pollination (Steiner 1998) and pollination through pseudocopulation resulting from sexual deceit (Paulus and Gack 1990, 1992; Paulus 1998). Nevertheless, the exploration of the distribution and diversity of specialised beetle pollination in the Orchidaceae evidently is still in its initial stages.

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