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Keeping the golden mean: plant stiffness and anatomy as proximal factors driving endophytic oviposition site selection in a dragonfly

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ABSTRACT

Oviposition site selection is a crucial component of habitat selection in dragonflies. The presence of appropriate oviposition plants at breeding waters is considered to be one of the key habitat determinants for species laying eggs endophytically. Thus, *Lestes macrostigma*, a species which is regarded as threatened in Europe because of its highly disjunct distribution, typically prefers to lay eggs in the sea club rush *Bolboschoenus maritimus*. However, little is known about how the anatomical and mechanical properties of plant tissues determine the choice of *L. macrostigma* females. We examined green shoots of six plant species used by *L. macrostigma* for oviposition, either in the field (actual oviposition plants) or under experimental conditions (potential oviposition plants), to analyse anatomical and mechanical properties of shoots in a framework of known preferences regarding plant substrates for oviposition. As expected, the anatomy of shoots differed between representatives of two plant families, Cyperaceae and Juncaceae, most essentially in the distribution of supporting bundles and the presence of large aeriferous cavities that may affect egg placing within a shoot. The force necessary to puncture the tested plant samples ranged from 360 to 3298 mN, and their local stiffness ranged from 777 to 3363 N/m. We show that the shoots of *B. maritimus*, the plant most preferred by *L. macrostigma*, have intermediate characteristics regarding both the stiffness and specific anatomical characteristics. The bending stiffness of the ovipositor in *L. macrostigma* was estimated as 1414 N/m, one of the highest values recorded for zygopteran dragonflies so far. The ecological and behavioural implications of plant choice mechanisms in *L. macrostigma* are discussed in the context of the disjunct distribution of this species.

1. Introduction

Oviposition, and the related habitat selection, is a keystone of insect fitness because adult choice is likely to influence the survival of their progeny (Williams and Feltmate, 1992). Habitat selection has been extensively studied in phytophagous insects laying eggs on the plant surface (e.g., Thompson and Pellmyr, 1991; Tilmon, 2008). In contrast, less attention has been paid to omnivorous and carnivorous insects (e.g., Benedict et al., 1983; Griffin and Yeargan, 2002) but also to insects laying eggs into plant tissues, a very ancient reproductive strategy (Béthoux et al., 2004). The proximal factors driving oviposition site selection in these species are foliage toughness, tissue thickness and trichome density, which provide clues regarding desiccation risk, accessibility to trophic resources, predation risk, etc. for their progeny (e.g., Pasquier-Barre et al., 2000, 2001; Griffin and Yeargan, 2002; Lundgren et al., 2008).

Dragonflies (Odonata) constitute an order of exclusively carnivorous insects, and the group of so-called 'endophytic' dragonflies oviposit within different living and dead plant tissues (Corbet, 2004). The vegetation composition is regarded to be an important distal cue for adults when they select larval habitat (Buskirk and Sherman, 1985; Michiels and Dhondt, 1990; Guillermo-Ferreira and Del-Claro, 2011). Especially in endophytic species, both oviposition site selection and production of an egg clutch consist of three successive steps (Lutz and Pittman, 1968; Waage, 1987; Martens, 2001; Matushkina and Lambret, 2011; Lambret et al., 2015a,b): (i) after copulation, the flying tandem or the female alone searches for a site to land for oviposition ('initial preference'); (ii) the female touches the plant surface with her ovipositor to obtain information on the potential insertion site, and tries to penetrate the

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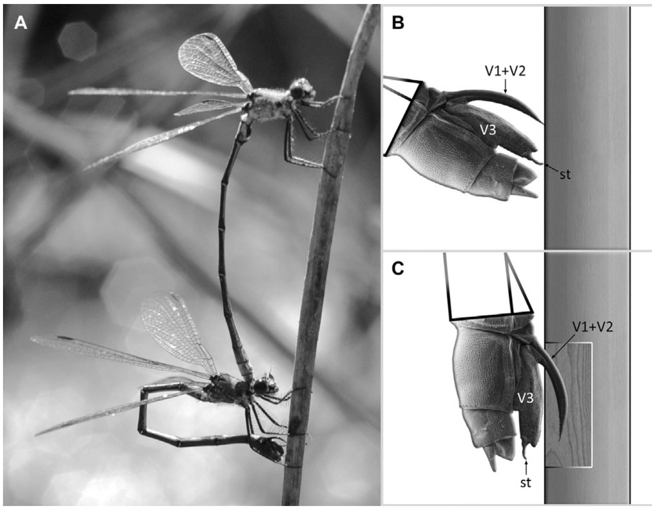

Fig. 1. *Lestes macrostigma* laying eggs. (A) Lateral view of the tandem with female ovipositing in a dead shoot of *Juncus maritimus*. (B) Schematic representation of the ovipositor touching the plant surface; note the contact of the cutting valves and stylus with the plant surface. (C) The cutting valves of the ovipositor have penetrated into the plant tissues. Abbreviations: st, stylus; V1 + V2, cutting valves; V3, sheathing valve.

plant substrate by alternately moving her ovipositor cutting valves ('ovipositor insertion') (Fig. 1); (iii) after the cutting valves have been entirely inserted, the female starts to lay eggs into the prepared hole ('egg deposition').

Dragonflies are well known to have substrate preferences during proximal oviposition site selection (e.g., Martens, 2001; Srivastava et al., 2005). There is evidence regarding the advantages that these preferences can provide to their progeny (e.g., Siva-Jothy et al., 1995; Rantala et al., 2004). On the other hand, it is not yet clearly understood which mechanisms and proximal factors the dragonfly female uses to evaluate the suitability of a plant substrate for egg deposition. Grunert (1995) stated that, among other factors, the stiffness of plant tissues may be important in oviposition substrate selection. The time required to penetrate plant tissues with the ovipositor can vary widely depending on the plant species (Lambret et al., 2015b) and we presume that the stiffer the tissue, the longer the time required. This may cause various effects, from reduced number of eggs in a clutch to increased risk of disturbance by conspecifics and predation. There is a direct correlation between the stiffness of the female ovipositor and the stiffness of their preferred plant substrates (Matushkina and Gorb, 2007). In other words, there are purely mechanical restrictions limiting the use of stiffer plant substrates by endophytic dragonflies. However, very soft plant materials also seem to be avoided by dragonflies (Grunert, 1995). This suggests that the female's decision to deposit eggs is not only based on the successful penetration of plant tissues but also on other proximal factors, such as plant anatomy. However, the influence of plant anatomy on the dragonfly's oviposition decision has not been explored hitherto.

Hence, in the present study we aimed to reveal possible relations between the physical characteristics of oviposition plant substrates and their level of preference during oviposition site selection in one dragonfly species. We focused on *Lestes macrostigma* (Eversmann, 1836) (Zygoptera, Lestidae), a species whose larvae mainly inhabit slightly brackish, temporary ponds, a disappearing and fragmented habitat along the Mediterranean coast (Dijkstra and Lewington, 2006; Zacharias and Zamparas, 2010). This species is particularly interesting because it is considered *nearly threatened* and *vulnerable* at the Mediterranean and the European scale, respectively (e.g., Riservato et al., 2009; Kalkman et al., 2010), and even *vulnerable* to *critically endangered* in some places (Lambret et al., 2009; Bence et al., 2011). *L. macrostigma* is also a relevant model species, since adult insects are known to have plant substrate preferences which directly and indirectly influence their progeny's survival (Lambret et al., unpublished data). Indeed, the vegetation of its typical habitats includes several types of oviposition substrates (living and dead shoots of various plant species). Although oviposition behaviour is observed in many of them, egg deposition only takes place in some of them (e.g., Martynov and Martynov, 2007; Lambret et al., 2015a,b). Living shoots of *Bolboschoenus maritimus* (Cyperaceae) and dead shoots of *Juncus maritimus* (Juncaceae) are clearly preferred during the different steps of oviposition (Lambret et al., 2015a,b). Living shoots of *J. maritimus* and dead shoots of *Juncus acutus* (Juncaceae) are less preferred. All the above-mentioned substrates can be regarded as 'actual substrates'. Egg deposition in living shoots of *Schoenoplectus tabernaemontani*, *Schoenoplectus litoralis* and *Eleocharis palustris* (Cyperaceae) has only been observed under laboratory conditions and never in the field, so that these types of shoots are regarded as 'potential substrates' (Lambret and Matushkina, unpublished observations). Egg deposition in living shoots of *J. acutus* has never been observed (Lambret et al., 2015b).

In the present study, we characterise the mechanical properties of six living plant species used by *L. macrostigma* for egg deposition, either in the field (actual oviposition plants) or under experimental conditions (potential oviposition plants). The anatomy of these plant species was studied to reveal possible correlations between the structure of the plant tissues and their mechanical properties. Additionally, the mechanical properties of the *L. macrostigma* ovipositor were investigated in a separate experiment.

2. Materials and methods

2.1. Plant sampling

In the early morning of 23 June 2010 intact plants with roots were collected from the National Natural Reserve of the Marais du Vigueirat, a protected area of the Camargue (southern France), at several brackish temporary swamps where *L. macrostigma* reproduces (43°30′N; 04°48′E). On the same day, the living plants were transferred to the Department of Functional Morphology and Biomechanics at Kiel University (Germany) for subsequent investigation. During transportation, plants were kept in wet impermeable cover at ca. 15 °C. Plant investigations lasted ca. 2 weeks and during this period plants were kept at room temperature and indirect sunlight in boxes filled with natural water. Only living green shoots with no signs of decay were tested.

Nine oviposition substrates from six plant species were examined: (1) younger light green and (2) older brownish-green shoots of *B. maritimus*; (3) younger light green and (4) older dark green shoots of *J. maritimus*; (5) younger light green and (6) older dark green shoots of *J. acutus*; green shoots of (7) *S. litoralis*, (8) *S. tabernaemontani*, and (9) *E. palustris*. Although dead shoots can be preferred oviposition substrates, they were not tested because their stiffness appears to be strongly influenced by the environmental characteristics, such as humidity and temperature. Each substrate was represented by 3–4 individual plants.

2.2. Plant anatomy

Plant shoots were dissected in thin transverse sections using a razor blade and investigated under a Zeiss Axioplan microscope in a glycerol drop. Reflected white light was used to study the general anatomy of plant dissections. Ultraviolet light regime with the Zeiss fluorescein filter set (BP 450–490, FT 510, and LP 520; Carl Zeiss Axi-