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# **Sexual dimorphism and sexual conflict in the diving beetle *Agabus uliginosus* (L.) (Coleoptera: Dytiscidae)**

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## Abstract

Sexual conflict can drive intersexual arms races, with female resistance and male persistence traits coevolving antagonistically. Such arms races are well documented in some diving beetles, although how widespread sexual conflict is in this family remains unclear. The European dytiscid *Agabus uliginosus* has a strikingly dimorphic female; individuals from most regions being smooth and male-like, but those from some populations having a strongly roughened dorsum, a trait which has attracted the name *dispar*. We demonstrate that rough and smooth females differ consistently in the development of dorsal surface microreticulation, and that these females are associated with males which differ in the development of their persistence traits. These findings extends the occurrence of pre-insemination sexual conflict and associated intrasexual dimorphism in Dytiscidae, suggesting that such mating systems are relatively widespread in these beetles.

## Introduction

Sexual conflict can drive evolutionary “arms-races” between males and females, resulting in the development of striking sexual dimorphism (Parker, 2006). In many diving beetles (Dytiscidae) males have modified, sucker-like articulo-setae on the tarsi of their fore- and middle legs which have been demonstrated to increase their ability to grasp females during mating (Aiken & Khan, 1992). In contrast, females of some species may have enhanced dorsal sculpture, which functions as an anti-grasping device during pairing (Karlsson Green *et al.*, 2013). Such traits are concentrated in the larger Dytiscinae, where pre-insemination sexual conflict dominates the sexual system (Miller & Bergsten, 2014a). Whilst these large diving beetles provide one of the best-studied examples of sexual antagonism in animals, the phylogenetic distribution of these traits, and the number of times such mating systems have evolved in the diving beetles as a whole remain poorly understood.

Some dytiscids are also intrasexually dimorphic, with two forms of female differing in their resistance traits; some being rough, others smooth, like males (Miller, 2003; Miller & Bergsten 2014a). Differences in female resistance appear to drive the evolution of counter-modifications in male attachment devices, with non-random mating between male and female morphs leading to linkage disequilibrium between male and female traits and the coexistence of morphs through negative frequency dependent selection (Härdling & Bergsten, 2006; Härdling & Karlsson, 2010). In some species there is pronounced geographical variation in the relative frequencies of rough and smooth female morphs, although the drivers of these distributions remain poorly understood (Karlsson Green *et al.*, 2014). In a handful of cases, such as *Hydroporus memnonius* Nicolai (Bilton *et al.*, 2008), rough and smooth female populations are almost entirely allopatric, with virtually no overlap in nature.

In *Hydroporus memnonius* rough and smooth females are associated with morphologically distinct males, differing in the size, number and distribution of adhesive articulo-setae on their tarsi (Bilton *et al.*, 2008). Males from matt

female populations possess more, individually larger suckers than those associated with smooth females, an observation suggestive of sexual antagonism, despite the relatively short pairings which appear characteristic of Hydroporinae (Miller, 2003). Here we report on both inter and intrasexual dimorphism in the diving beetle *Agabus uliginosus* (L.), which also has allopatric smooth and rough female populations; the latter being referred to the (now) var. *dispar* (Bold). We explore the nature and extent of morphological differentiation between smooth and rough females, and whether males from different female populations differ in their persistence traits.

## Materials and methods

*Agabus uliginosus* is a widespread European species, occurring from Britain and Spain east to the Urals (Nilsson & Petrov, 2006; Bilton, 2010). Despite this the species is relatively rare, being found in natural seasonal waterbodies and has declined following agricultural intensification. Matt females are known from Britain, the Netherlands, Denmark and Sweden, being fixed in populations and allopatric to the widespread male-like form (Foster *et al.*, 2016). Specimens were collected from localities listed in Table 1 using a D-framed net (1 mm mesh), killed with ethyl acetate and preserved in 70% ethanol.

Beetles were air-dried overnight then mounted onto metal stubs using double-sided carbon tape, in preparation for electron microscopy. Males were mounted ventral side uppermost to expose the underside of tarsi; females mostly mounted dorsal side uppermost to allow study of microsculpture. In some cases male legs were removed and mounted beside the specimen using rapid drying silver paint, to reduce flare. Typically the right tarsi were imaged, although if these were damaged/absent, tarsi from the left side were substituted. All specimens were gold sputter coated using an Emitech K550 Coating Unit, and photographed in a JEOL JSM5600LV Scanning Electron Microscope. Pronotal and elytral microsculpture were imaged at x 160 and x 1,000, with a photo being taken on the pronotal disc and the left elytral shoulder region at the higher magnification. Tarsi were photographed at x

220, at least two images being taken per tarsus (to capture all setae), with one front and one middle tarsus being photographed per beetle, and care taken to ensure at least one photo imaged the basal tarsal segment directly from below for width measurement. Structures were quantified from photos by eye and using ImageJ 1.46r. In the case of females, a transverse line of 200  $\mu\text{m}$  was drawn across each photo in ImageJ, and the area of all microreticulation polygons which crossed this line estimated, along with the width of inter-polygon channels. In males, the maximum width of the basal tarsomere of fore and middle legs was estimated using ImageJ. The number of articulo-setal suckers on male tarsi were quantified by eye in ImageJ. Any setae the beetle had lost during life were included in this count, as there position could be identified by the presence of empty insertion pits (e.g. bottom right of Fig. 2p). In this species articulo-setae occur on the three basal segments of fore and mid tarsi, in a tightly packed field, making it impossible to identify which tarsal segment many individual setae arise from. As a consequence we counted the total number of setae on each leg, rather than attempting to allocate setae to individual segments. The area of five sucker plates per tarsus per beetle was measured using the polygon tool in UTHSCA Image Tool version 3.0.

*t* or Mann-Whitney *U* tests were used to determine whether the microreticulation of smooth females and var. *dispar* differed in polygon area and width of inter-polygon channels. Repeated measures ANOVAs were used to explore whether males associated with the two forms differed in tarsal width, the number of articulo-setae, and the size of sucker terminal plates. All statistical tests were conducted in SPSS version 21.

## Results

Females of the two forms differed significantly in surface microsculpture of both pronotum and elytra; smooth females resembling males, *dispar* females having a distinctly matt appearance (Fig. 1a). Pronotal microreticulation of var. *dispar* females was composed of smaller polygons than smooth females (mean area =  $570.6 \mu\text{m}^2 \pm 184.7\text{SD}$  vs.  $1313.3 \mu\text{m}^2 \pm 491\text{SD}$ ;  $t = -4.49$ ,  $p < 0.001$ ,  $\text{DF} = 16$  – Fig. 1b, Fig. 2a-b vs. e-f). Matt females also had

significantly smaller polygons in their elytral microreticulation (mean area  $242.7 \mu\text{m}^2 \pm 123.3\text{SD}$  vs.  $773.4 \mu\text{m}^2 \pm 182.1\text{SD}$ ;  $W = 323$ ,  $p = 0.001$  – Fig. 1b, Fig. 2c-d vs. g-h) with individual polygons more tumid in appearance in the matt form (compare Fig. 2 c and g). Inter-polygon channels in *dispar* were significantly broader than those of smooth females, on both the pronotum (mean width  $2.1 \mu\text{m} \pm 0.6\text{SD}$  vs.  $0.75 \mu\text{m} \pm 0.25\text{SD}$ ;  $t = 8.72$ ,  $p < 0.001$ ,  $DF = 33$  – Fig. 1c, Fig. 2a-b vs. e-f) and elytra (mean width  $3.0 \mu\text{m} \pm 0.9\text{SD}$  vs.  $0.70 \mu\text{m} \pm 0.20\text{SD}$ ;  $t = 10.3$ ,  $p < 0.001$ ,  $DF = 28$  – Fig. 1c, Fig. 2c-d vs. g-h). Male pronotal and elytral sculpture (Fig. 2i-l) was identical to that of smooth females.

Male tarsal widths (Fig. 1d, Fig. 2m-p) differed significantly between the two forms, *dispar* population males having wider tarsi than those from smooth female populations (ANOVA  $F_{1,32} = 20.52$ ,  $p < 0.001$ ). Tarsal widths did not differ significantly between fore and mid legs in either form. Male fore and mid tarsi supported dense brushes of articulo-setal suckers on the three basal segments (Fig. 2m-p), with conical shafts and ellipsoid terminal plates. These setae were entirely absent from the tarsi of females (Fig. 2q-r). Males from matt form populations had significantly more suckers on their tarsi than those from smooth female localities (ANOVA  $F_{1,32} = 27.57$ ,  $p < 0.001$  – Fig. 1e, 2m-n vs. o-p). The mid tarsi supported significantly more suckers than the fore tarsi in both forms (ANOVA  $F_{1,32} = 13.58$ ,  $p = 0.001$ ). The terminal plates of individual suckers varied in size from ca.  $270$  to  $1,120 \mu\text{m}^2$ , most being above  $600 \mu\text{m}^2$ . Sucker plates were not, however, divisible into two or more distinct size classes, there instead being a gradation in size on individual tarsi (e.g. Fig. 2m-p). Males associated with *dispar* females had larger sucker plates on their fore-tarsi than those from smooth female populations (mean area  $881 \mu\text{m}^2 \pm 141\text{SD}$  vs.  $781 \mu\text{m}^2 \pm 102.5\text{SD}$ ; ANOVA  $F_{1,6} = 8.14$ ,  $p = 0.029$ ). Sucker plates on mid tarsi did not differ significantly in area between the two forms, although those from males associated with *dispar* females were slightly larger (mean areas  $778$  and  $781 \mu\text{m}^2$ , respectively).

## Discussion

Rough and smooth female populations of *Agabus uliginosus* differ dramatically in the development of their dorsal surface microreticulation and these females are associated with morphologically distinct males. Such observations are strongly indicative of a sexually antagonistic arms race in this *Agabus*, escalation of female resistance traits in *dispar* populations apparently being matched by an intensification of male persistence abilities.

The matt *dispar* and smooth female forms differ consistently in the structure of their pronotal and elytral microreticulation. In *dispar* the channels of the reticulation network are wider than in the smooth female form, and these channels enclose much smaller meshes, particularly on the elytra. Whilst the function of surface microreticulation remains unclear, it is a consistent feature of the cuticle of beetles including many dytiscids (Balfour-Browne 1940; Crowson 1981). Similar dimorphisms in dorsal reticulation are seen in females of a number of hydroporines, including *Hydroporus memnonius* (Bilton *et al.* 2008) and *Hygrotus impressopunctatus* Schaller (Karlsson Green *et al.* 2014), but this is the first time they have been documented in Agabinae, which occupies a more basal phylogenetic position (Miller and Bergsten 2014b).

The more intense reticulation of *dispar* females will reduce the adhesive ability of male sucker setae (see Karlsson Green *et al.* (2013) and may represent a means by which females could reduce the costs associated with multiple matings, or exert greater choice during encounters with males (Miller and Bergsten 2014a). Specifically, the smaller microreticulation meshes, with wider channels between them may increase water flow into attached male suckers, reducing both attachment time and suction force (see Bergsten and Miller 2007).

Males associated with *dispar* females have broader fore and mid tarsi than their counterparts from smooth female populations. These broader tarsi support a more articulo-setal suckers, a male counter measure likely to increase persistence ability on a rough surface (e.g. Bergsten *et al.* 2001).



The area of terminal plates of individual male suckers is, on average, approximately half that of the microreticulation meshes on the pronotum of smooth females, and approximately the same size as the meshes of their elytra, meaning that many suckers may attach without their suction being impeded by a channel. In contrast, the microreticulation meshes in *dispar* females are considerably smaller than mean sucker area, particularly on the elytra. Despite male *uliginosus* possessing suckers as small as  $270\ \mu\text{m}^2$ , the response to *dispar* females has not involved a shift to smaller sucker size, or more small suckers, in an attempt to match the size of reticulation meshes. Indeed, as noted above, as well as being more numerous overall, sucker plates on the fore tarsi are significantly larger in males associated with this form. Stork (1980) demonstrated that the pulling force exerted by the tarsi of *Chrysolina polita* (L.) increases with the number of adhesive setae present. Nilsson (1986) suggested that smaller and more numerous tarsal suckers should be advantageous for males attaching to rough females in larger dytiscids. In the case of *Graphoderus zonatus verrucifer* Sahlberg Bergsten *et al.* (2001) found a higher number of small suckers in males from populations with abundant granulate females, but also noted that the three largest protarsal suckers were larger in these individuals. Here we find a similar increase in the size of large suckers, and their number, changes presumably relating to the mechanics of attachment to the surface of *dispar* females.

Our findings extend the phylogenetic distribution of pre-insemination sexual conflict and intrasexual dimorphism in diving beetles. Such mating systems are now well documented in the Dytiscinae (e.g. Miller 2003) and Hydroporinae (Bilton *et al.* 2008; Karlsson Green 2014) to which we can add the more basal Agabinae. Species like *Agabus uliginosus*, where two distinct forms of female occur in allopatry, may prove particularly illuminating in understanding the evolutionary outcomes of such interactions in nature. Males associated with *dispar* females may have a mating advantage with either female form, as they would be expected to be better able to attach on both rough and smooth surfaces (Bergsten and Miller 2007), perhaps facilitating expansion of *dispar* at the expense of the smooth form.

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## Figure Legends

**Figure 1.** Differences between *Agabus uliginosus* morphs. a, habitus of smooth female, *dispar* and male. b, area of female microreticulation polygons. c, width of female microreticulation channels. d, male tarsal widths. e, male tarsal sucker number. grey and black in b-e indicate smooth female and *dispar* respectively, bars represent means (+SD).

**Figure 2.** Microreticulation and tarsi of *Agabus uliginosus*. a, e, i; b, f, j, pronotal reticulation. c, g, k; d, h, l, elytral reticulation. a-d, *dispar* females. e-h, smooth females. i-l males. m, o, q fore and n, p, r mid tarsi. m-n *dispar* population male. o-p smooth population male. q-r Female.