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Male clasping ability, female polymorphism and sexual conflict: fine-scale elytral morphology as a sexually antagonistic adaptation in female diving beetles

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During sexual conflict, males and females are expected to evolve traits and behaviours with a sexually antagonistic function. Recently, sexually antagonistic coevolution was proposed to occur between male and female diving beetles (Dytiscidae). Male diving beetles possess numerous suction cups on their forelegs whereas females commonly have rough structures on their elytra. These rough structures have been suggested to obstruct adhesion from male suction cups during mating attempts. However, some diving beetle species are dimorphic, where one female morph has a rough elytra and the other has a smooth elytra. Here, we used biomechanics to study the adhesive performance of male suction cups on the female morphs in two diving beetle species: *Dytiscus lapponicus* and *Graphoderus zonatus*. We compared adhesion on the rough and the smooth female morphs to infer the function of the rough elytral modifications. We found that the adhesive force on the rough structures was much lower than on other surfaces. These findings support the suggestion of sexual conflict in diving beetles and a sexually antagonistic function of the rough female structures. In addition, males differed in their adhesive capacity on different female surfaces, indicating a male trade-off between adhering to smooth and rough female morphs.

1. Introduction

During sexual conflict, arising from sexually antagonistic selection during mating or parental care [1], males and females are expected to evolve traits or behaviours that enforce the fitness interests of one sex at a disadvantage for the opposite sex [1,2]. Sexually antagonistic traits might, for example, increase or decrease mating rate [2]. Pairs of such sexually antagonistic traits are expected to coevolve in response to each other and could theoretically result in a perpetual arms race between males and females [1,3,4]. In spite of much interest in such arms races driven by sexual conflict, they are still only known to occur in a very few cases [5–7], and they may not be an inevitable or even common outcome of sexual conflicts in Nature [8,9].

In several insect species experiencing sexual conflict, males possess 'grasping traits' which they use to hold a female before, during or after mating [2]. While male grasping traits are quite well studied, there is in general little knowledge of corresponding female morphological adaptations [10]. A notable exception to this lack of examples comes from water striders, where males have organs that aid in grasping a female during mating and females have corresponding genital spines that repel males and thus serve to increase female control in mating interactions [11,12]. Another suggested pair of antagonistic
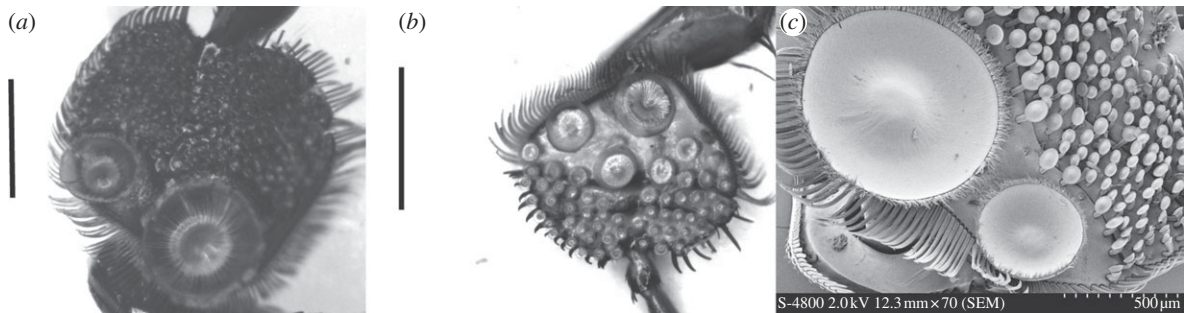
Figure 1. Male protarsal palette with suction cups. (*a*) *Dytiscus lapponicus* (light microscopy), (*b*) *Graphoderus zonatus* (light microscopy), (*c*) detail of suction cups in *D. lapponicus* (scanning electron microscopy). Scale bars, (*a,b*) 1 mm. Scale bar in the bottom right corner in (*c*) denotes 500 μm.
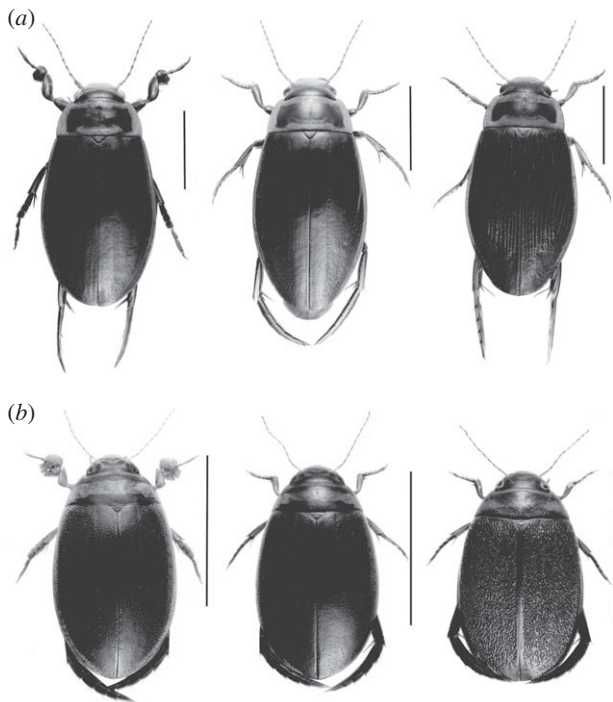

Figure 2. Species used in the study. From left to right in each panel: male, smooth female morph, rough female morph. Scale bars, 10 mm. (*a*) *Dytiscus lapponicus* and (*b*) *Graphoderus zonatus*. Photographs courtesy of Johannes Bergsten.

Figure 3. Frequencies of the smooth and the rough morphs in natural populations. Black bar indicates frequency of smooth morph and white bar indicates frequency of rough morph. Populations used in the current study are indicated with an asterisk above the bars. (*a*) *Dytiscus lapponicus* populations and (*b*) *Graphoderus zonatus* populations.

traits is the suction cups of male diving beetles (Dytiscidae) and the rough modifications on female beetles' elytra [13–15], whose interplay we address in this study.

Males of several diving beetle species, and in some species also females (e.g. genus *Hydroporus* [16]), have adhesive setae on the ventral side of their forelegs [13,15,17]. In subfamily Dytiscinae, these adhesive setae are found only in males and formed as suction cups (figure 1). Males use these suction cups to capture a female and hold her during mating, which proceeds when the female is exhausted after a long pre-mating struggle [18,19]. Furthermore, in many Dytiscinae the male inserts a mating plug in the female genitalia to prevent her from mating with other males [18,19]. In several Dytiscinae females have structural modifications on their elytra, e.g. furrows or granules (figure 2). Darwin [20] recognized these rough female structures in the genus *Acilius*, and interpreted them as aiding males to hold on to females during mating. More recently, however, it has been proposed that the male suction cups, on the contrary, would not easily adhere to rough surfaces [13] and that these rough structures thus may increase female control during mating. This behavioural setting and these morphological structures in females indicate the existence of differential fitness interests of the sexes.
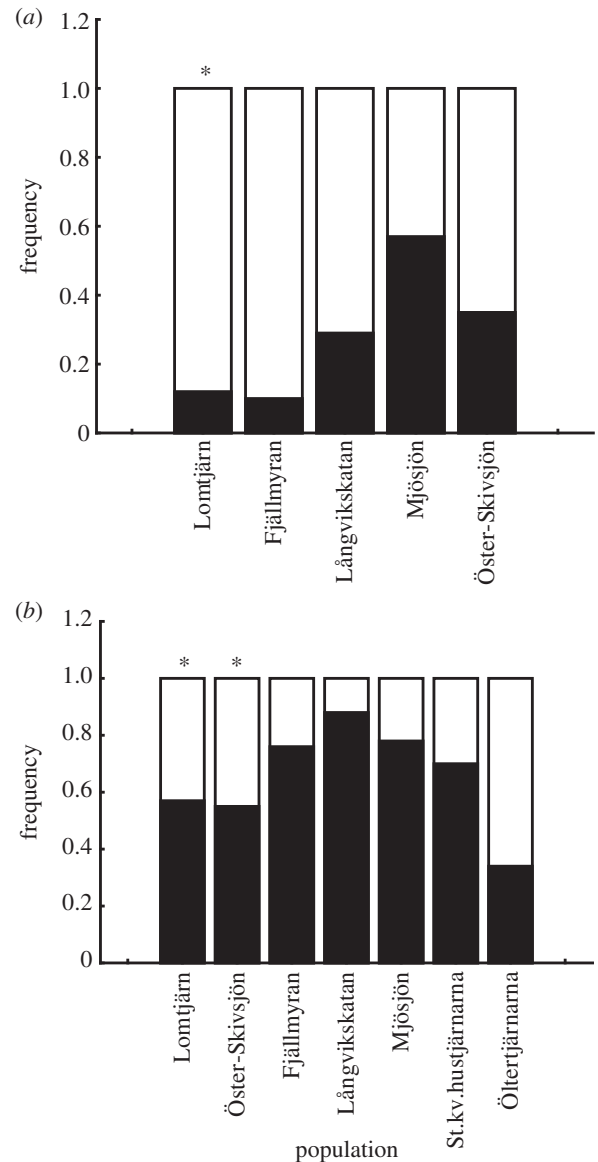
In the genus *Acilius*, male suction cups and female dorsal modification are phylogenetically correlated with each other; an increased dorsal sculpturing in females is followed by an increased differentiation between large and small suction cups on male protarsi [13]. A loss of female dorsal sculpturing is furthermore followed by a reversal of the male suction cup morphology [13]. Thus, this phylogenetic pattern is strongly

Table 1. The number of individuals from each species and population used, and the surfaces on which adhesion was measured, as well as the method (sensor operated by manipulator or manually).

species	population	number of individuals	surfaces and method
Dytiscus lapponicus	Lomtjärn	6	glass surface, manipulator operated
			glass surface, manually operated
Graphoderus zonatus	Lomtjärn	6	male elytra, manually operated
			male pronotum, manually operated
			rough female elytra, manually operated
G. zonatus	Öster-Skivsjön	5	rough female pronotum, manually operated
			smooth female elytra, manually operated
			smooth female pronotum, manually operated

suggesting sexually antagonistic coevolution between these traits [13] and, accordingly, a sexual conflict over mating has recently been suggested to operate in diving beetles [14,15].

In some diving beetle species, however, two female morphs coexist within local populations; one with a smooth elytron (the 'smooth' morph) and one with a rough elytron (the 'rough' morph) [17] (figure 2). The rough structures seem to have dominant inheritance over the smooth surface [21] and the selection regimes responsible for maintaining this polymorphism are not fully understood (K. Karlsson Green, E. I. Svensson, J. Bergsten, R. Härdling & B. Hansson 2010, unpublished data).

Here, we studied the adhesive forces of male suction cups on smooth and rough female morphs in two species of diving beetles: *Dytiscus lapponicus* (Gyllenhal) (figure 2a) and *Graphoderus zonatus* (Hoppe) (figure 2b). Our aim was to understand the function of the rough female structures by comparing the male adhesive properties on the two female morphs. The adhesive strength of male suction cups has previously been measured for the diving beetle *Dytiscus alaskanus*, but only on an artificial surface of glass [22]. To our knowledge, the actual adhesive performance of suction cups on the surfaces of female morphs has not been quantified before.

2. Material and methods

2.1. Study species

Members of the family Dytiscidae, diving beetles, are found in freshwaters across the globe, with some species also inhabiting brackish waters [17]. We conducted our study on two species of diving beetles: *D. lapponicus* and *G. zonatus*. *D. lapponicus* (figure 2a) is a rather large diving beetle (24.1–30.0 mm), which occurs from Great Britain and Fennoscandia to West Siberia [17]. *Graphoderus zonatus* (figure 2b) is a smaller beetle (12.0–15.7 mm), which is widespread from central Fennoscandia to Siberia [17]. In both species, female dimorphism occurs with one female morph having a smooth elytron, to the human eye perfectly resembling the males, while the other morph has rough structures on the elytron (figure 2).

In *D. lapponicus* the rough female morph has 10 longitudinal furrows in the elytron [17] (figure 2a), while in *G. zonatus* the rough females are granulated [17] (figure 2b). In both species, the two female morphs coexist within populations (figure 3). Males of both species have numerous suction cups on their pro- and mesotarsi. The three basal segments of the protarsi are modified into a big palette containing suction cup-like adhesive setae. The protarsal palette of *D. lapponicus* consists of
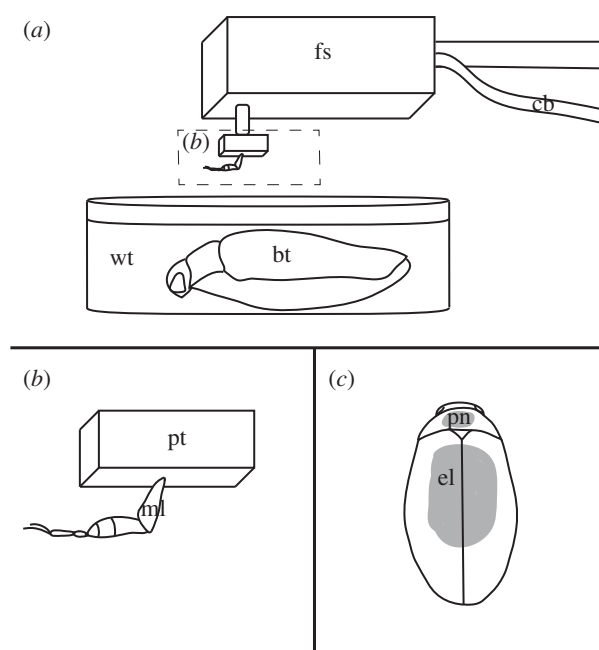

Figure 4. Schematic drawing of the set-up for adhesion measurements. (a) Experimental set-up. The male leg bearing suction cups was mounted on a plastic cube attached to the force sensor. Beetles, on which dorsal surface adhesion was measured, were mounted under water. The force sensor was moved downwards to the dorsal side of the beetle until the suction cups came into contact with the dorsal surface and adhered. (b) Close up of the mounted male foreleg. (c) Dorsal side of the beetle, the dorsal surface of which was probed. Measurements were taken approximately on the shadowed areas of the pronotum (pn) and the elytra (el). The figure also shows: plastic tube (pt), force sensor (fs), cable connection to the amplifier (cb), male foreleg (ml), Petri dish filled with water (wt), beetle body firmly attached to the bottom of the Petri dish (bt).

two large and numerous (on average 228) small suction cups (figure 1a), while male *G. zonatus* have three larger and, on average, 55 smaller cups (figure 1b) (K. Karlsson Green 2010, unpublished data). In *G. zonatus*, the suction cup composition is related to the relative frequencies of the two female morphs in the local population: a higher frequency of the rough female morph is associated with an enlarged protarsal palette as a whole, as well as three enlarged suction cups and also many smaller and more numerous suction cups, in males [14]. This suggests that the number, size and composition of male suction cups are adapted to female elytral structure, with certain characteristics being favoured when the rough female morph is common in a local population. The range of morph frequencies
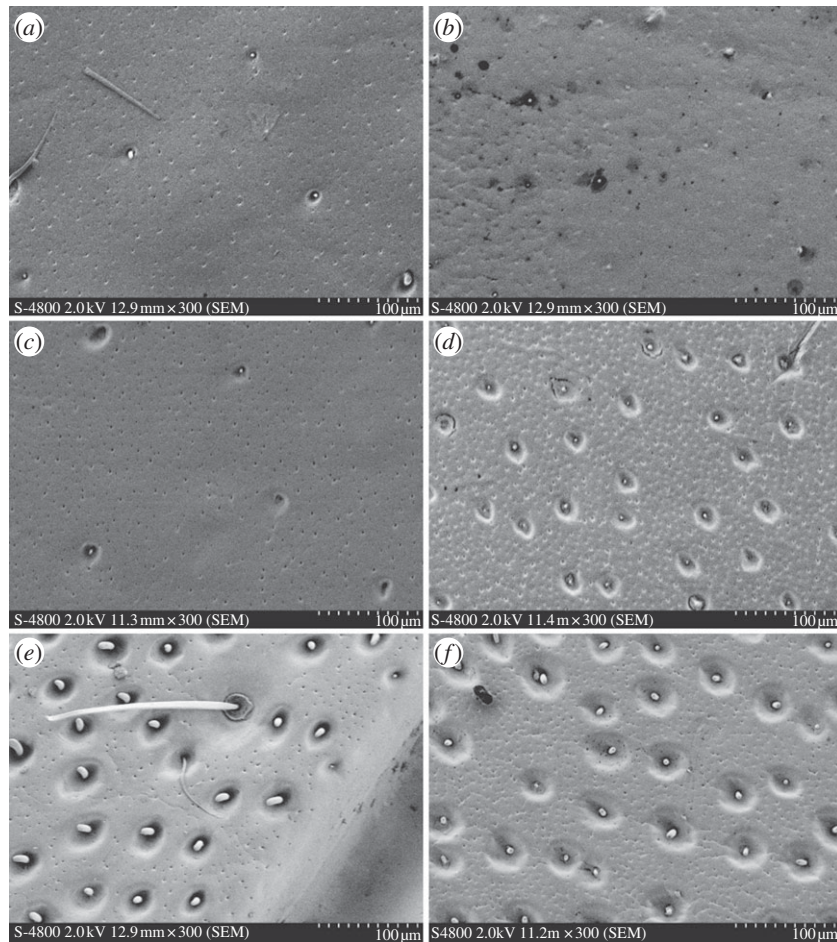
Figure 5. SEM images of *Dytiscus lapponicus*. Elytral surfaces (*a,c,e*) and pronotal surfaces (*b,d,f*). Scale bars are given in the bottom right corner of each micrograph. (*a*) Male elytron, (*b*) male pronotum, (*c*) smooth female elytron, (*d*) smooth female pronotum, (*e*) rough female elytron and (*f*) rough female pronotum.

for the two species in this study is presented in figure 3. We studied the protarsal suction cups (figure 1), which have a more complex composition of suction cups and which are crucial for the initial capture of females. A male will initially attempt to adhere to a female anywhere on her dorsal surfaces with his front legs, while the female rapidly swims away to escape in the bottom substrate [18]. If the male manages to hold on to the female and to manoeuvre her to the correct mating position, mating may proceed [18]. For this initial capture and for the manoeuvring of the female, the protarsal suction cups are probably more important than the mesotarsal suction cups [14,18,22].

2.2. Fieldwork and handling of the diving beetles

Diving beetles were captured from northern Sweden in September 2009. We used *D. lapponicus* from Lake Lomtjärn (latitude, 63.81636; longitude, 20.37118) and *G. zonatus* from both Lake Lomtjärn and Lake Öster-Skivsjön (latitude, 63.87029; longitude, 20.51157) (table 1). Beetles were captured either with traps that were baited with fish and left overnight, or by active netting along the shoreline of ponds. The diving beetles were housed in small jars; initially filled with lake water from the field, and later with dechlorinated tap water. They were given an ad libitum diet in the form of red chironomid larvae. Individuals that were used in the adhesion study were killed using chloroform.

2.3. Scanning electron microscopy

To elucidate the microstructure of the different beetle surfaces, we studied the beetle surfaces using a scanning electron microscope (SEM). We also studied the suction cups from males of both species. Pieces of the elytron, pronotum and suction cups

of individuals from both female morphs and a male from both species were fixed in 99% alcohol and dried in the air for 24 h. Afterwards, the samples were sputter-coated with gold–palladium (10 nm) and visualized using a cryo-SEM Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) at room temperature. The accelerating voltage was 2 and 4 kV.

2.4. Adhesion measurements

The adhesive capacity of male suction cups was measured under water with a load cell force transducer (25 g capacity; World Precision Instruments, Sarasota, FL). The sensor motion was controlled manually or with a motorized three-axis manipulator (DC3001R; World Precision Instruments, Sarasota, FL). The signal from the force sensor was amplified, digitalized and recorded using a MP100WSW data acquisition system (Biopac Systems Inc., Goleta, CA) and the software AcqKnowledge v. 3.7.0. The interaction force between the suction cups and various surfaces was registered.

We used intact beetles as surfaces (figure 4*a*). These beetle surfaces came from individuals that belonged to the same species and population as the focal males. The same surface individuals were used for all males within the same population. Surface individuals were horizontally mounted with dental wax in a large Petri dish (figure 4*a*). The Petri dish was filled with water, immersing the whole beetles.

For the males from which the suction cups were used, the right front leg was cut off at the first segment. The leg was glued with a mixture of beeswax and rosin to a small (approx. $2 \times 2 \times 5$ mm) plastic block (figure 4*b*). This was done so that the palette, on which the suction cups are located, was fixed horizontally and could move freely. When attaching the leg to the plastic block,
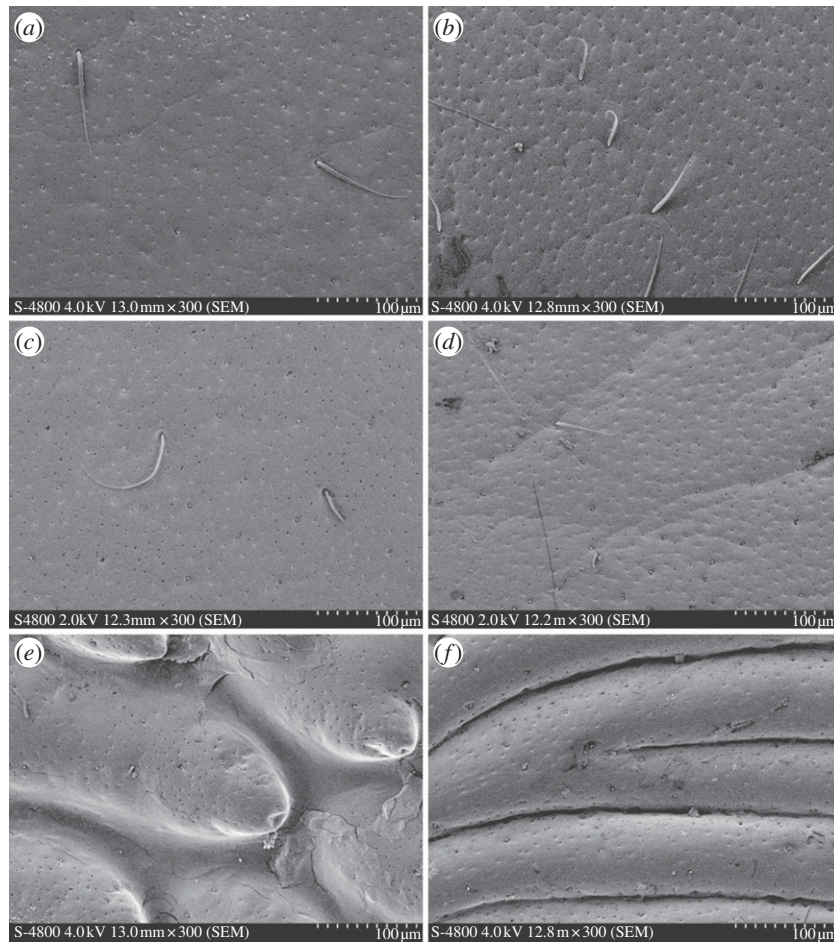
Figure 6. SEM images on *Graphoderus zonatus*. Elytral surfaces (*a,c,e*) and pronotal surfaces (*b,d,f*). Scale bars are given in the bottom right corner of each surface figure. (*a*) Male elytron, (*b*) male pronotum, (*c*) smooth female elytron, (*d*) smooth female pronotum, (*e*) rough female elytron and (*f*) rough female pronotum.

the cut end of the leg was at the same time sealed with wax and thus evaporation from the leg was prevented. Finally, the plastic block was fixed onto the sensor with dental wax (figure 4*a*). We measured adhesion on the elytron and the pronotum of a male and of the two female morphs (figure 4*c*). We thus measured adhesion on six different beetle surfaces: male elytron, male pronotum, rough female elytron, rough female pronotum, smooth female elytron and smooth female pronotum (table 1).

As the cover wings of diving beetles are not flat but slightly rounded it was difficult to obtain adhesion when the sensor was manoeuvred by a manipulator, which only moves strictly vertically and thus may not reach the beetle surface perpendicularly. Therefore, we took all measurements manually. During the manual measurements, the sensor was manoeuvred slowly, and as steadily as possible, up and down, allowing the suction cups on the mounted palette to adhere to the surface individuals. To evaluate the method, we measured adhesion on glass both manually and with the manipulator. Thus, we obtained eight different treatments in total for our analyses (table 1). On each surface, we measured adhesion (expressed as the pull-off force in newtons, N) with 30 repetitions for each male leg and used five or six males per population (table 1). For the manipulator measurement, we changed position on the glass three times to study the impact of site for adhesive capacity. When we measured adhesion manually, however, the sites changed with every measurement. To avoid an effect of usage, we randomized the order of the surfaces that we measured between males, although we always started by measuring with the manipulator on glass to obtaine a reference point of adhesion. All experiments were carried out under ambient conditions and under a 10 mm layer of water. Our data is deposited in the Dryad data repository [23].

2.5. Statistical analyses

We used all the 30 repetitions for each male, i.e. each male contributed to several different measurements in these analyses. To ensure statistical independence and to avoid pseudoreplication owing to multiple measurements from each male subject, we incorporated male ID as a random factor in all our analyses and thus performed mixed models. To correct for any usage effect during the experiments, i.e. that the suction cups would be worn during the measurements, we also incorporated the number of measurements (order) as a covariate in all analyses. Each population was analysed separately.

Initially, we tested for differences between the measurements using manipulator and manual measurements by comparing adhesion on glass obtained with the two methods (glass manipulator and glass manually) for each species. To do this, we performed analysis of covariance (ANCOVA) for each species with adhesion force as the dependent variable and method (i.e. manipulator or manually), male ID (random effect), loading force (covariate) and order (covariate) as independent variables. We incorporated only the main effects and no interactions between them in this first analysis, as the interactions were not considered to be of major interest at this initial stage.

Next, we studied the effect of site of male attachment on adhesion force, i.e. if the particular location on the surface affects adhesion. As the site slightly differs between each repetition with the manual method, and as there are probably different factors affecting adhesion with this method, we analysed the effect of site only for the manipulator-operated measurements on glass. Thus, we used ANCOVAs with adhesion force as the dependent variable and site, male ID (random effect), loading force (covariate) and order (covariate) as independent variables. Also in this

Table 2. Adhesion of male tarsi on the six different surfaces. *p*-values were obtained from ANCOVAs. Adhesion is the dependent variable and male ID, surface, loading force and order are independent main factors.

source	effect	*D. lapponicus*	*G. zonatus* (Lomtjärn)	*G. zonatus* (Öster-Skivsjön)
male ID	random	$F_{1,5} = 3.96$	$F_{1,5} = 7.04$	$F_{1,4} = 4.30$
		$p = 0.005**$	$p < 0.001***$	$p = 0.006**$
		SS = 0.00517	SS = 0.00186	SS = 0.00069
surface	fixed	$F_{1,5} = 24.72$	$F_{1,5} = 4.29$	$F_{1,5} = 3.97$
		$p < 0.001***$	$p = 0.003**$	$p = 0.006**$
		SS = 0.03251	SS = 0.00104	SS = 0.00078
loading force	fixed	$F_{1,1} = 3.42$	$F_{1,1} = 13.70$	$F_{1,1} = 18.23$
		$p = 0.097$	$p = 0.009**$	$p = 0.011*$
		SS = 0.00040	SS = 0.00017	SS = 0.00087
order	fixed	$F_{1,1} = 0.98$	$F_{1,1} < 0.01$	$F_{1,1} = 3.62$
		$p = 0.323$	$p = 0.97$	$p = 0.057$
		SS = 0.00008	SS < 0.00001	SS = 0.00005
male ID * surface	random	$F_{1,25} = 9.35$	$F_{1,25} = 13.32$	$F_{1,20} = 10.05$
		$p < 0.001***$	$p < 0.001***$	$p < 0.001***$
		SS = 0.01868	SS = 0.00419	SS = 0.00255
male ID * loading force	random	$F_{1,5} = 3.96$	$F_{1,5} = 0.99$	$F_{1,4} = 4.06$
		$p = 0.005**$	$p = 0.42$	$p = 0.003**$
		SS = 0.00517	SS = 0.00006	SS = 0.00021
surface * loading force	fixed	$F_{1,5} = 5.60$	$F_{1,5} = 1.19$	$F_{1,5} = 5.35$
		$p < 0.001***$	$p = 0.31$	$p < 0.001***$
		SS = 0.00224	SS = 0.00008	SS = 0.00034
error		d.f. = 1032	d.f. = 1032	d.f. = 859
		SS = 0.08251	SS = 0.01298	SS = 0.01091
adjusted R^2		0.65	0.59	0.53

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

analysis, we only incorporated the main effects as interactions were considered to be of minor biological interest.

Third, we performed ANCOVAs to explore the differences in adhesion on the different beetle surfaces. We had adhesion force as the dependent variable and surface, male ID (random effect), loading force (covariate) and order (covariate) as independent factors. Here, we constructed models that included interactions between the main factors (surface×male ID) as well as the interactions involving the factors and the covariate (surface×loading force, male ID×loading force). The three-way interaction and any interaction with order were excluded, as they were not considered to be meaningful or interesting from a biological perspective. We used Tukey's post hoc test to compare if and how the different surfaces differed from each other in their adhesion force.

Finally, we investigated individual differences in male capacity to adhere to the rough and the smooth elytra. We explored this using a similar model to that described above that included surface, male ID (random effect), loading force (covariate) and order (covariate) as independent factors. We included interactions between the main factors as well as between each main factor and loading force. Here, we were especially interested in the male ID×surface interactions. Such a significant interaction would indicate that the adhesive performance of individual males differs between female morphs in a unique way for different males. That is, some males would do better than others on the smooth morph and other males would do better on the rough morph. All statistical analyses were performed in the software STATISTICA (Statsoft, Inc., 2004).

3. Results

The fine-scale structures of the beetle surfaces are illustrated with SEM images (figures 5 and 6). Distinct differences between the elytral surfaces of the two morphs were revealed (figures 5c,e and 6c,e). Also the pronota of the two morphs differ, although not so remarkably as the elytral surfaces (figures 5d,f and 6d,f). The pronotum and elytra of males are smooth in both morphs (figures 5a,b and 6a,b).

When comparing the two different methods for measuring adhesion (manual and using a manipulator), for *D. lapponicus*, we found that all main effects were significant (method: $F_{1,115} = 54.16$, $p < 0.001$; male ID: $F_{1,115} = 29.03$, $p < 0.001$; loading force: $F_{1,115} = 18.63$, $p < 0.001$; order: $F_{1,115} = 8.53$, $p = 0.004$). The manual and manipulator-operated methods differed for this species, with the manual method producing an overall higher adhesion (mean (s.e.): manual, 0.04 N (0.0006); manipulator, 0.03 N (0.006)). For the Lomtjärn population of *G. zonatus*, the two methods did not significantly differ from each other (method: $F_{1,233} = 0.71$, $p = 0.402$; male ID: $F_{3,233} = 118.50$, $p < 0.001$; loading force: $F_{1,233} = 55.22$, $p < 0.001$; order: $F_{1,233} = 11.94$, $p < 0.001$). For the Öster-Skivsjön population of *G. zonatus* all included effects were significant (method: $F_{1,292} = 47.66$, $p < 0.001$; male ID: $F_{4,292} = 49.82$, $p < 0.001$; loading force: $F_{1,292} = 4.61$, $p = 0.033$; order: $F_{1,292} = 21.31$, $p < 0.001$). Thus, the methods

differ also in this population, and again the manual method generated the greater adhesion (mean (s.e.): manual, 0.017 N (0.0003); manipulator, 0.014 N (0.003)).

The particular site on the test surface affected adhesion for *D. lapponicus* (site: $F_{2,112} = 9.04$, $p < 0.001$; male ID: $F_{3,112} = 187.92$, $p < 0.001$; loading force: $F_{1,112} = 1.055$, $p = 0.307$; order: $F_{1,112} = 30.76$, $p < 0.001$). For both the Lomtjärn and the Öster-Skivsjön populations of *G. zonatus*, however, site did not affect adhesion (*G. zonatus* Lomtjärn, site: $F_{2,112} = 0.95$, $p = 0.388$; male ID: $F_{3,112} = 102.85$, $p < 0.001$; loading force: $F_{1,112} = 25.14$, $p < 0.001$; order: $F_{1,112} = 0.29$, $p = 0.59$; *G. zonatus* Öster-Skivsjön, site: $F_{2,141} = 1.45$, $p = 0.238$; male ID: $F_{4,141} = 49.79$, $p < 0.001$; loading force: $F_{1,141} = 1.27$, $p = 0.263$; order: $F_{1,141} = 6.92$, $p = 0.009$).

In all three populations, we found that the pull-off force was affected by two of the main factors: male ID and surface (table 2). Loading force had a significant effect in the two *G. zonatus* populations and was not significant in *D. lapponicus* (table 2). Order was non-significant in the Lomtjärn population of *G. zonatus* and in *D. lapponicus*, while it was very close to significance in the Öster-Skivsjön population of *G. zonatus* (table 2). Of particular interest in terms of differential clasping ability of individual males was that, in all populations, the male ID×surface interactions were significant (table 2). This means that individual males differed in their adhesive capacity on different female surfaces, suggesting the potential for trade-offs between different female morphs.

Adhesion was lower on the rough female surfaces than on the smooth female surfaces in all three populations (figure 7). Adhesion on the rough elytra and on the rough pronotum did not differ in *D. lapponicus* and in the Öster-Skivsjön population of *G. zonatus* (figure 7a,c). In the Lomtjärn population of *G. zonatus* adhesion on these rough surfaces differed among the surfaces, with the least adhesion on the rough elytra (figure 7b). Although the quantitative results differed slightly between our three study populations, the overall finding was that the rough elytra and the rough pronotum generated the least adhesion force while, in most cases, adhesion on the male and on the smooth female surfaces were similar to each other (figure 7).

The separate analyses of differences in adhesion on the rough and the smooth elytra revealed that the male ID×surface interaction was significant in all three populations (table 3 and figure 8). This means that individual males clearly and significantly differed in their capacity to adhere to rough and smooth surfaces. Even if all males perform worse on the rough elytra, males that perform better on the rough surface did not necessarily also perform better on the smooth elytra, i.e. the rank order changed among males (figure 8). These results and significant interactions involving male ID (table 3) suggest that there is a clear potential for a trade-off in males between male clasping ability on smooth and rough female morphs (figure 8).

4. Discussion

Diving beetles have recently been proposed to represent a case of sexual conflict, with a sexually antagonistic coevolution between male suction cups and female elytral modifications [13–15]. In the current study, we investigated the adhesive performance of male suction cups (figure 1) on females to infer the function of the rough structures on female elytra (figures 2, 5 and 6). Our results revealed that these rough female structures
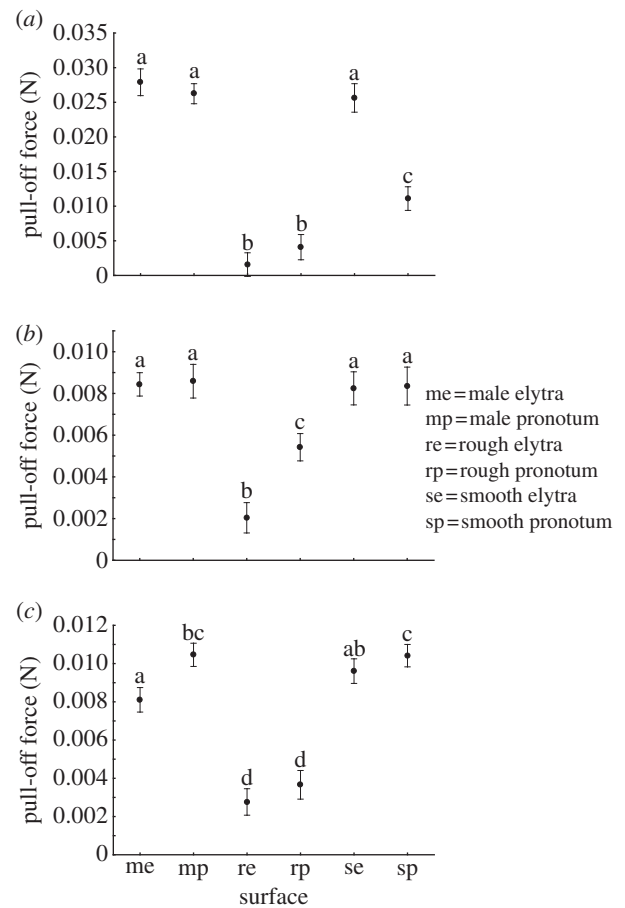

Figure 7. Average adhesion (pull-off force) on the different beetle surfaces for the three populations. Different letters indicate statistically significant differences in adhesion force between treatments (ANCOVA, Tukey's post hoc test; see also table 1). Note the different scales on the *y*-axes in (*a*–*c*). (*a*) *D. lapponicus*, (*b*) *G. zonatus* (Lomtjärn population) and (*c*) *G. zonatus* (Öster-Skivsjön population).

obstruct male adhesion. Thus, the study does support the interpretation of the rough structures as a sexually antagonistic trait with an anti-grasping function. These findings further strengthen the suggestion that past antagonistic interactions between males and females have favoured the development of sex-specific conflict traits in this insect group (i.e. suction cups and dorsal modifications, respectively).

The adhesive strength of male suction cups was considerably lower on both the rough elytra and the rough pronotum than on the other surfaces (figure 7). The larger *D. lapponicus* generates stronger adhesion on the smooth surfaces. In contrast, on the rough surfaces, the two beetle species perform more or less equally, generating forces of only around 0.002–0.004 N. It is interesting to note that the adhesion generated on the rough elytra and the rough pronota was similar in two of the populations (figure 7), despite the structural difference between the surfaces captured in the SEM figures (figures 5 and 6). It may be that a minor structural roughness is enough to decrease adhesion substantially, although a lack of difference in adhesion between these surfaces may be due to our method (see below). The structural difference in the pronotum (figures 5 and 6) indicates, however, that these surfaces are also important in antagonistic interactions. Alternatively, pronotum sculpturing might simply be a correlated evolutionary response in females to selection for increased sculpturing on the elytra.

In any case, there may be a strong role for sexual conflict in shaping the fine-scale elytral morphology in females. There is,

Table 3. Adhesion of male tarsi on the rough and the smooth elytra. p-values were obtained from ANCOVAs. Adhesion is the dependent variable and male ID, surface, loading force and order are independent main factors.

source	effect	D. lapponicus	G. zonatus (Lomtjärn)	G. zonatus (Öster-Skivsjön)
male ID	random	$F_{1,5} = 0.71$	$F_{1,5} = 0.94$	$F_{1,4} = 0.58$
		$p = 0.630$	$p = 0.519$	$p = 0.690$
		SS = 0.00090	SS = 0.00043	SS = 0.00016
surface	fixed	$F_{1,1} = 39.01$	$F_{1,1} = 6.66$	$F_{1,1} = 3.32$
		$p < 0.001^{***}$	$p = 0.042^*$	$p = 0.124$
		SS = 0.01033	SS = 0.00052	SS = 0.00017
loading force	fixed	$F_{1,1} = 6.26$	$F_{1,1} = 2.65$	$F_{1,1} = 40.44$
		$p = 0.018^*$	$p = 0.140$	$p < 0.001^{***}$
		SS = 0.00031	SS = 0.00003	SS = 0.00028
order	fixed	$F_{1,1} = 1.01$	$F_{1,1} = 3.40$	$F_{1,1} = 1.74$
		$p = 0.315$	$p = 0.066$	$p = 0.188$
		SS = 0.00008	SS = 0.00003	SS = 00.00002
male ID * surface	random	$F_{1,5} = 8.26$	$F_{1,5} = 30.97$	$F_{1,4} = 23.96$
		$p < 0.001^{***}$	$p < 0.001^{***}$	$p < 0.001^{***}$
		SS = 0.00328	SS = 0.00143	SS = 0.00087
male ID * loading force	random	$F_{1,5} = 0.38$	$F_{1,5} = 1.15$	$F_{1,4} = 0.70$
		$p = 0.864$	$p = 0.335$	$p = 0.591$
		SS = 0.00015	SS = 0.00005	SS = 0.00003
surface * loading force	fixed	$F_{1,1} = 1.22$	$F_{1,1} = 0.03$	$F_{1,1} = 15.38$
		$p = 0.269$	$p = 0.874$	$p < 0.001^{***}$
		SS = 0.00001	SS < 0.00001	SS = 0.00014
error		d.f. = 340	d.f. = 340	d.f. = 283
		SS = 0.02704	SS = 0.00314	SS = 0.00258
adjusted R^2		0.68	0.71	0.64

$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

however, in general limited knowledge of the morphological traits in females [10,24] perhaps because behavioural adaptations [2,10] or tolerance to mating attempts [9] evolve more easily. Another reason might be that other morphological adaptations evolve which may have an antagonistic function although they do not interfere with the male grasping traits *per se*. For example, in damselflies (genus *Ischnura*), some female morphs have evolved a male-like coloration as an adaptation to reduce visual detection by males and hence avoid interference with males [25–27]. Although data on the female and male fitness consequences of carrying or interacting with these morphological structures in diving beetles are lacking, and would be needed in future experiments, our data provide independent support from biomechanical first principles to the sexual conflict hypothesis. The use of independent evidence from biomechanics is a strong research programme in evolutionary biology to infer adaptation, sometimes denoted 'the argument from design' [28–30]. Testing biomechanical predictions derived from sexual conflict models is probably an underestimated research approach that could complement other, more traditional ecological, behavioural and phylogenetic approaches to infer adaptation [31]. Such a biomechanical approach may also be fruitful in other systems when studying potential morphological anti-grasping adaptations in females.

Our results suggest that there is probably strong selection on males to overcome female morphological adaptations to achieve successful mating, and recent phylogenetic studies have shown that male suction cup morphology changes following changes in the female dorsal structures [13]. However, the presence of two female morphs may counter the evolution of a single male adaptation to the antagonistic female traits. In *G. zonatus*, there is a correlation between male suction cup morphology and female morph frequencies in local populations [14], indicating that the relative presence of morphs affects suction cup morphology. It is quite probable that males experience conflicting selection between adhering to the smooth and adhering to the rough structures. Interestingly, in all analyses, the male ID × surface interaction was significant (tables 2 and 3). Even if all males perform worse on the rough elytra, males that perform better on the rough surface did not necessarily also perform better on the smooth elytra, that is, the rank order changed among males (figure 8). Hence, individual males clearly differed in their adhesive capacity on different female surfaces, indicating a potential trade-off in male clasping ability between smooth and rough female morphs.

Conflicting selection between adhering to the smooth and adhering to the rough structures may be reflected in the suction cup morphology. Indeed, the morphology of male suction cups correlates with the female morph frequencies in local
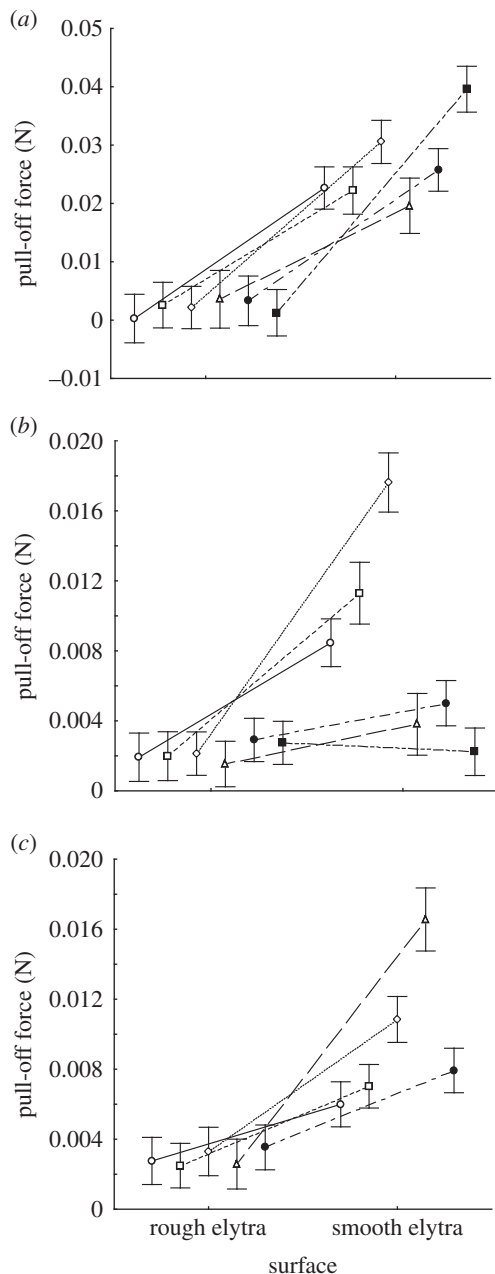
Figure 8. Adhesion (pull-off force) of individual males on the rough and smooth elytra (connected by lines), respectively. (a – c) Illustrate the interaction male ID × surface, which was significant according to the ANCOVAs in all the populations (see also table 1). Note the different scales on the y-axes in the three panels. (a) *D. lapponicus*, (b) *G. zonatus* (Lomtjärn population) and (c) *G. zonatus* (Öster-Skivsjön population).

populations [14], indicating that the presence of different elytral structures affects the male suction cup morphology (and vice versa). As a parallel to another adhesive system in insects, males of the Colorado potato beetle (*Leptinotarsa decemlineata*) seem to experience a similar trade-off between the capacity to adhere to smooth females during mating and the capacity to adhere to rough surfaces of plants during locomotion [32]. It is known from experiments with Colorado potato beetles that small flexible adhesive hairs have stronger adhesion on rough surfaces than larger and less flexible ones that are mainly adapted for adhesion on smooth surfaces [32]. We may assume that, in the diving beetles, large suction cups are an adaptation to the smooth surfaces, whereas the numerous little ones may enhance adhesion to the uneven surfaces.

Considering the theoretical models on how mating polymorphisms could be maintained through sexual conflict, one outcome could be that males end up evolutionarily trapped between different genetic female morphs, and not being well adapted to any of them [33]. Another evolutionary outcome of such sexually antagonistic interactions could be the formation of two more or less discrete male clusters with distinct suction cup phenotypes (i.e. male morphs), which would be well adapted and specialized to one female morph each. The diving beetle *Hydroporus memnonius* presents a scenario in which the two female morphs are always associated with one distinct male morph each, with the male morphs having different suction cup morphologies [16]. *Hydroporus memnonius* morph pairs are almost always allopatric, a pattern which in this case may be the result of replacement by the more favourable morph rather than of sexual conflict dynamics [16]. Recent theory further suggests that two pairs of morphs that mate assortatively can lead to frequency-dependent sexual conflict dynamics that also maintains genetic polymorphism within populations [34,35].

Our results do not contradict the possibility that the elytral modifications of females have additional ecological functions. In the diving beetle *Agabus bipustulatus*, both males and females have dorsal microreticulation and there is a positive relation between reticulation and altitude [36]. In this species, it has been suggested that the dorsal structures might serve as protection from dangerous UV radiation at the higher altitudes [36]. Yet other diving beetle species show a strong geographical pattern in the frequencies of the different female morphs [16,17]. If similar environmental correlations exist in the two species studied here, a combination of natural and sexual selection may be operating on these elytral structures. Thus, external ecological factors and natural selection may interact with selection from more internal frequency-dependent processes in local populations that are driven by mating interactions and sexual conflict.

We note that our adhesion measurements are likely to be subject to some methodological noise. When we measured adhesion manually, we were not able to manually manoeuvre the sensor exactly as with the manipulator (see §3). This might have contributed to the discrepancy between the manual and manipulator-operated measurements on glass. The higher adhesion obtained with the manual method may be the result of the higher retraction speed for manual measurements. These methodological issues might also explain why the trade-off in adhesive ability for individual males was not even more pronounced. However, our main result—that adhesion is lower on the rough surfaces—appears to be robust given the very large differences between the rough and the other surfaces found in all three populations (figure 7).

To our knowledge, this is the first quantitative study of male adhesion on the actual female surfaces in these diving beetles. We provided experimental confirmation of the previously suggested antagonistic function of these dorsal modifications, as they obstruct adhesion from male suction cups [13,15,37]. These results suggest that a signature of sexual conflict can also be found at the micro-morphological level, which is consistent with previous findings of male mating structures of other insects [38–40]. Our study emphasizes the important role of an experimental approach, here combining microstructural studies with force measurements, when estimating performance on morphological structures involved in sexual conflict [31]. Further exploration of adhesive capacity to the

rough females might also be of future practical interest for the development of artificial adhesive systems, with implications for the fields of biomimetics and bioinspiration [41].

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