

# What is going on between aposematic carabid beetles? The case of *Anchomenus dorsalis* (Pontoppidan 1763) and *Brachinus sclopeta* (Fabricius 1792) (Coleoptera Carabidae)

T. ZETTO BRANDMAYR<sup>1,4</sup>, T. BONACCI<sup>1</sup>, A. MASSOLO<sup>2,3</sup> and P. BRANDMAYR<sup>1</sup>

<sup>1</sup> Dipartimento di Ecologia, Università della Calabria, Via P. Bucci, I-87036 Arcavacata di Rende (CS), Italy

<sup>2</sup> Dipartimento di Biologia Animale e Genetica, Università di Firenze, Via Romana 17, I-50125 Firenze, Italy

<sup>3</sup> EULABS, European Laboratories ([www.eulabs.eu](http://www.eulabs.eu))

Received 30 April 2006, accepted 9 June 2006

Interspecific aggregations among insects are quite common, but not among carabid beetles. Moreover, aggregations of aposematic species, and morphologically similar beetles such as *Anchomenus dorsalis* and *Brachinus sclopeta* are even rarer. Most interestingly, in these aggregations, we observed a peculiar rubbing behaviour of *A. dorsalis* towards *B. sclopeta*. In this study we aimed (i) to verify if *A. dorsalis* displays the rubbing behaviour towards non-aposematic species, (ii) to describe in detail and to quantify this behaviour between *A. dorsalis* and *B. sclopeta*, and (iii) to verify if previous experience of inter-specific gregariousness with the same species may exert some effects on the frequency of the rubbing display.

We have investigated the qualitative and quantitative characteristics of rubbing behaviour towards aposematic and non-aposematic carabid beetle species, and then we used *A. dorsalis* and *B. sclopeta* as model species. We tested the rubbing of *A. dorsalis* towards *B. sclopeta* with either solitary *A. dorsalis* or in *A. dorsalis* coming from monospecific (*A. dorsalis*) or from heterospecific (*A. dorsalis* and *B. sclopeta*) aggregations. We finally tested the effectiveness of the *B. sclopeta* cuticular odour in eliciting rubbing from *A. dorsalis* by presenting olfactory dummies (special paper) either soaked with water or after it had previously been placed in a monospecific group of *B. sclopeta* for 15 days.

First of all, we found important differences in the occurrence of rubbing behaviour towards aposematic and non-aposematic species, as *A. dorsalis* displayed the rubbing only towards the former. Secondly, rubbing frequency depended on the pre-test conditions (isolated, mono- or heterospecific aggregations) of the tested *A. dorsalis* individuals, as no rubbing occurred in individuals coming from heterospecific aggregations. The paper presentation tests demonstrated that the odour of *B. sclopeta* was sufficient to provoke rubbing in *A. dorsalis*.

---

<sup>4</sup> Corresponding author: Tullia Zetto-Brandmayr (Tel. +39984492977, E-mail address: [tuzetto@unical.it](mailto:tuzetto@unical.it)).

We conclude that aggregation in these species of carabid beetles may influence their anti-predatory defences possibly increasing the effectiveness of the aposematic (warning) signals.

More work is needed to delineate in detail the evolutionary significance of this behaviour, focusing on the possibility that interspecific aggregations may not be so rare in ground beetles, and that this positive interaction could involve a larger number of species and genera, at least in wet, sun-exposed and open land habitat types.

KEY WORDS: gregariousness, *Brachinus sclopeta*, *Anchomenus dorsalis*, rubbing behaviour.

---

Introduction . . . . .	336
Material and methods . . . . .	338
Results . . . . .	340
Discussion . . . . .	345
References . . . . .	346

## INTRODUCTION

Interspecific aggregation is common in many species of insects, particularly in more or less ephemeral environmental patches (SEVENSTER 1996, SEVENSTER & VAN ALPHEN 1996, HEARD & REMER 1997, KRIJGER & SEVENSTER 2001, WOODCOCK et al. 2002 in HUTTON & GILLER 2004). Spatial and temporal variation in insect aggregations may be due to variation in the presence of aggregation or disaggregation cues or to variation in the receptivity of the animal to a particular cue or suite of cues (RATCHFORD & EGGLESTON 2000). Strong interspecific aggregation has been identified in subtropical (GILLER & DOUBE 1989 in HUTTON & GILLER 2004) and temperate (FINN & GILLER 2000 in HUTTON & GILLER 2004) dung beetles when presented with artificial or natural dung pads respectively. Interspecific aggregation was found in the carrion flies *Phoenicia coeruleiviridis* and *Phormia regina* (IVES 1991), lady beetles *Hippodamia convergens* and *H. tredicimpunctata* (LEE 1980) and locust nymphs *Schistocerca gregaria* and *Locusta migratoria migratorioires* (NIASSY et al. 1999). Many species form overwintering aggregation for extended periods (up 10 months; LEE 1980). This dormancy period is believed to synchronize the reproductive and feeding stages of the beetles with their food (HODEK 1973 in LEE 1980).

In gregarious insects the individual risk of being attacked decreases as the number of conspecifics increases (SILLÉN-TULLBERG & LEIMAR 1988, "dilution" effect). The "dilution" effect (BERTRAM 1978 in SILLÉN-TULLBERG & LEIMAR 1988) does not require usually cooperative behaviour, both in intra- and interspecific aggregations.

Interspecific aggregation in carabid beetles are known for *Anchomenus dorsalis* (syn. *Agonum dorsale*) and some species of the genus *Brachinus*. BEDEL (1895-1914 in ZABALLOS 1985) refers to interspecific aggregation between *Chlaenius* spp., *Agonum* spp., *Poecilus* spp. and some species of *Brachinus*. THIELE (1977) reports aggregations between *Nebria brevicollis* and *Agonum dorsale*, *Nebria brevicollis* and *Brachinus crepitans*, and between *Dromius* spp. and *Brachinus* spp. WAUTIER (1970, 1971) reported aggregation between *B. sclopeta* and *B. explodens*.

The interspecific aggregation of *A. dorsalis* and *Brachinus* species are frequent (BEDEL 1881, MÜLLER 1926, JEANNEL 1942, MAGISTRETTI 1965, JEANNE 1968, NOVOA 1975, BONACCI et al. 2004).

The adaptive significance of carabid beetle interspecific aggregation may be related to antipredatory strategies, possibly exerted by a dilution effect, but in some cases undefended species may profit from being together with defended ones (i.e. those that are poisonous, distasteful, or that have organs for spraying dangerous substances).

All the species of the genus *Brachinus* show an aposematic colouration, possibly to signal that they are protected against predators by means of a chemical reaction triggered by the predator's attack behaviour. The beetles of the genus *Brachinus* have a double chambered apparatus, where hydroquinones are converted in quinones which are violently sprayed against the disturbing source, accompanied by an explosion and a high amount of heat (EISNER & MEINWALD 1966; ANESHANSLEY et al. 1969; EISNER 1970; SCHILDKNECHT 1970, 1975; EISNER & DEAN 1976). As a consequence, intraspecific aggregation may also enhance the chemical defences against predators in *Brachinus* species (EISNER & DEAN 1976).

In some cases, as for some *Brachinus* species, aggregations may be found between chemically defended species as well, suggesting that a reinforcement of the overall chemical defence may play a role, along with an increased dilution effect (WAUTIER 1970, 1971; BONACCI et al. 2004).

Several carabid species are frequently found along with *Brachinus* groups in Southern Italy (Calabria; BONACCI et al. 2004), but the most common is certainly *Anchomenus dorsalis* (JEANNEL 1942, MAGISTRETTI 1965, ZABALLOS 1985), which shares the same shelters under stones, especially in spring.

*A. dorsalis* is an aposematic coloured species, with of chemical defences (SCHILDKNECHT 1970), that shows a peculiar behaviour of rubbing towards other aposematic species; LINDROTH (1949) observed this behaviour when *A. dorsalis* specimens were kept together with *B. crepitans*: "At least in captivity *Brachynus* behaves passively ..., whereas *Agonum* shows strikingly "friendly" behavior. Often it positions itself next to *Brachynus* and ardently rubs its back and the sides of the prothorax and elytra fore and aft, like a cat, against various parts of the body of *Brachynus* ... during the cleansing ritual, *Brachynus* remains passive" (sic). In recent years, in laboratory conditions, this behaviour has been observed to be displayed by *A. dorsalis* also towards many other aposematic carabid species (*Brachinus* and *Chlaenius*, several species; unpublished data).

No precise explanations of this display has been provided, but LINDROTH's "cleansing ritual" hypothesis relates this behaviour to a sort of cleaning behaviour. This interpretation would be confuted if the rubbing behaviour were not displayed towards non-aposematic species, but only towards aposematic ones. Conversely, our hypothesis is that *rubbing* is more likely to be related to an anti-predatory strategy strengthening defence mechanisms (dilution effect, or increased chemical defence). As a consequence, rubbing should not be displayed towards non-aposematic species.

Furthermore, we hypothesize that the rubbing behaviour is related to some "transfer", possibly of chemical substances, and thus we expect that rubbing would be displayed in the very first encounters of heterospecific individuals only, then reducing as time passes, as if the "transfer" had been completed.

To describe the characteristics of this inter-specific behaviour we focused our attention on the interactions between *A. dorsalis* and *B. sclopeta*, that is a quite common aggregation in Southern Italy. These species usually aggregate under heavy

stones in open lands with sparse vegetation, such as pasturelands, croplands or in humid, sun exposed soils (BONACCI et al. 2004, MAZZEI et al. 2005, R. PIZZOLOTTO unpublished data).

Within this study we aimed (i) to verify if *A. dorsalis* displays the rubbing behaviour towards non-aposematic species, (ii) to describe in detail and to quantify this behaviour between *A. dorsalis* and *B. sclopeta*, and (iii) to verify if previous experience of inter-specific gregariousness with the same species may exert some effects on the frequency of the rubbing display.

## MATERIAL AND METHODS

### *Animals*

The carabid beetles (adults) employed in this study were collected by hand in the field in some habitats of the Calabrian cultivated landscape: Crati Valley, province of Cosenza, Southern Italy (latitude: 39°35'56"N; longitude: 16°15'48"E and elevation: 60 m a.s.l.).

The animals were reared in laboratory in plastic cages provided with soil from the collecting site and were fed on bits of cow meat, pieces of earthworms or crushed snails.

They were placed in monospecific groups (individuals belonging to the same species), (*A. dorsalis* N = 227, *B. sclopeta* N = 30); heterospecific groups (animals belonging to both species) (*B. sclopeta* N = 15 and *A. dorsalis* N = 30) and solitary individuals (*A. dorsalis* N = 15 and *B. sclopeta* N = 15). Each group was reared in plastic cages (30 × 22 × 20 cm), whereas the single individuals were each placed in separate glass containers (diameter: 5 cm, depth: 9 cm). In each container 4 cm of clayey soil were placed. The rearing conditions were: 22-24 °C, L/D:18/6. The typical experimental arena was a Plexiglas cage, 6 cm long and 6 cm large with a plaster substratum 1.5 cm deep.

Tests consisted in placing one "receiver species" in the arena and keeping it alone for 5 min, then adding an *A. dorsalis* individual and recording the behavioural events.

The study was carried out during spring 2005.

### *Experimental design*

(1) To test the occurrence of rubbing behaviour towards non-aposematic species we used *A. dorsalis* reared in monospecific groups and 5 individuals for each one of the 23 carabid beetle species used as control species (see Table 1). On the whole, a total number of 33 species have been selected using different criteria: (a) aposematism: we used both aposematic and non-aposematic species; (b) spatial compresence: species occurring in the same habitats (found in the same collecting sites) or species from a different habitat or from other sites where *A. dorsalis* was not present; (c) phylogeny; we used both phylogenetically related and unrelated species (Table 1). The phylogeny of carabids has been constructed taking into account the fundamental views of the main authors (BALL et al. 1998), and integrating some recent results from molecular phylogeny (MADDISON et al. 1999, OBER 2002).

(2) For the detailed description and quantification of rubbing behaviour we used both sexes of the species *B. sclopeta* and *A. dorsalis*.

Each test lasted 30 min. For each experimental condition 15 trials were performed.

The experiment was carried out at 20-22 °C, in full daylight or lit by a reading lamp.

The behaviour of the carabid beetle was filmed using digital video recording equipment (Panasonic Digital Video, NV-DA1EG) and analysed using the software The Observer® XT (Noldus Information Technology).

Table 1.

Phylogenetic relationships and habitat of the tested ground beetle species, markedly simplified. *Anchomenus* belongs to the large group of “higher” Carabidae known as Harpalinae, all *Brachinus* species to the Brachiniinae, a probable sister group of the former. Aposematic species are shown in bold front.

Taxon	Forests	Open habitats	Riverside and Wetlands
<i>Omopron limbatum</i> (Fabricius 1776)			■
<i>Carabus coriaceus</i> Linné 1758		■	
<i>Carabus lefebvrei</i> Dejean 1826	■		
<i>Nebria kratteri</i> Dejean 1831	■		
<i>Leistus spinibarbis</i> (Fabricius 1715)	■		
<i>Bembidion quadrimaculatum</i> (Linné 1761)			■
<b><i>Anchomenus dorsalis</i></b> (Pontoppidan 1763)		■	■
<i>Calathus circumseptus</i> Germar 1824		■	
<i>Calathus fracassii</i> Heyden 1908	■		
<i>Metapedius pantanellii</i> Fiori 1903		■	
<i>Poecilus cupreus</i> (Linné 1790)		■	
<i>Platysma nigrita</i> (Paykull 1790)	■		
<i>Steropus melas</i> (Creutzer 1799)	■	■	
<i>Pterostichus bicolor</i> Aragona 1830	■		
<i>Carterus rotundicollis</i> Rambur 1837		■	
<i>Ophonus incisus</i> (Dejean 1829)		■	
<i>Acinopus picipes</i> (Olivier 1795)		■	
<i>Harpalus distinguendus</i> (Duftschmid 1812)		■	
<i>Parophonus hispanus</i> (Rambur 1838)		■	
<i>Parophonus mendax</i> (Rossi 1790)		■	
<b><i>Diachromus germanus</i></b> (Linné 1758)		■	■
<i>Scybalicus oblongiusculus</i> (Dejean 1829)		■	
<i>Licinus silphoides</i> (Rossi 1790)		■	
<b><i>Chlaeniellus vestitus</i></b> (Paykull 1790)		■	■
<b><i>Chlaeniellus olivieri</i></b> (Crotch 1871)		■	■
<b><i>Chlaenius spoliatus</i></b> (P. Rossi 1792)		■	■
<b><i>Chlaenius velutinus</i></b> (Duftschmid 1812)		■	■
<b><i>Trychochlaenius chrysocephalus</i></b> (P. Rossi 1790)		■	■
<i>Callistus lunatus</i> (Fabricius 1775)		■	
<i>Dinodes decipiens</i> (L. Dufour, 1820)		■	
<i>Microlestes luctuosus</i> Holdhaus 1912		■	
<b><i>Brachinus crepitans</i></b> (Linné 1758)		■	■
<b><i>Brachinus psophia</i></b> Audinet-Serville 1821		■	■
<b><i>Brachinus italicus</i></b> (Dejean 1831)		■	■
<b><i>Brachinus sclopeta</i></b> (Fabricius 1792)		■	■

### List of experiments

Expt. 1 — *A. dorsalis* from the monospecific group versus *B. sclopeta* from the monospecific group (15 tests performed).

Expt. 2 — *A. dorsalis* from individual cages versus *B. sclopeta* from individual cages (15 tests).

Expt. 3 — *A. dorsalis* from heterospecific groups versus *B. sclopeta* from the monospecific group (15 tests).

Expt. 4 — *A. dorsalis* from heterospecific groups versus *B. sclopeta* from heterospecific groups.

Expt. 5 — *A. dorsalis* from monospecific group and one of control species (5 tests for every species).

Expt. 6 — The tests on the decrement of rubbing behaviour lasted 110 min consisting of 3 observation periods of 30 min each (T1, T2 and T3), separated by 10 min intervals. Each animal (from the monospecific groups) was tested once.

To investigate the nature of the stimulus that provokes rubbing behaviour in *A. dorsalis*, we tested 32 individuals coming from monospecific groups to examine whether they showed rubbing behaviour towards 4 pieces of an olfactory dummie (special paper) previously placed in a monospecific group of *B. sclopeta* for 15 days. Each test with the olfactory dummie ( $n = 4$ ) was performed using simultaneously 4 individual of *A. dorsalis* and 1 dummie concurrently. Control tests ( $n = 4$ ) used one piece of paper soaked in water with 4 individuals tested concurrently.

### Statistical analysis

To test for differences in the occurrence of rubbing behaviour, we considered the relative frequency of occurrence computed as the ratio of the number of rubbing events over the total amount of recorded events (scour + calm + moving).

Differences amongst the 3 groups of experimental animals (alone-reared, and hetero- or monospecific groups) were first tested by the Kruskal-Wallis test, and then pair-wise comparisons were performed with the Mann-Whitney test (SIEGEL & CASTELLAN 1988). Similarly, the Friedman test was used to verify the decrement in rubbing behaviour in 3 periods of 30 min each, while the Wilcoxon test was used to verify differences amongst different periods (multiple comparisons, SIEGEL & CASTELLAN 1988).

Multiple comparisons of different treatments were taken into account by correcting the  $\alpha$  level using the Dunn-Šidák significance level correction method:  $\alpha' = 1 - (1 - \alpha)^{1/k}$ , where  $k$  is the number of comparisons (SOKAL & ROHLF 1995); in our case, comparing the effect of the 3 different classes of treatment, the new significance levels were set to:  $\alpha_{0.05}' = 0.0169$ ,  $\alpha_{0.01}' = 0.0033$ ,  $\alpha_{0.001}' = 0.00033$ .

The Friedman test was used to verify the change in rubbing behaviour between successive times periods.

When direct computation was not possible, the probability level has been computed using a complete randomisation method (permutation or exact test;  $P_{exact}$ ) or by a Monte Carlo simulation based on a 10,000 sampled tables ( $P_{Monte Carlo}$ ) (MEHTA & PATEL 1996, GOOD 2000).

## RESULTS

### Rubbing towards non-aposematic species

Rubbing was displayed only towards aposematic and protected species ( $n = 10$ ), while it never occurred towards the other 23 species of non-aposematic carabid beetles.

*Description of the behavioural events*

Behavioural events which constitute the “cleansing ritual” as defined by LINDROTH (1949) are on the whole three for each species: search, rubbing and rest for *A. dorsalis* and scour, calm and moving for *B. sclopeta*, as drawn in Fig. 1.

The three events depicted for *A. dorsalis* and *B. sclopeta* are strictly dependent. The rubbing event is displayed only by *A. dorsalis* towards *B. sclopeta* and its frequency and duration depends on the behavioural response of *B. sclopeta*. *A. dorsalis* often rubs its own body on the body of *Brachinus* resting motionless.

The behavioural events displayed by *A. dorsalis* versus *B. sclopeta* individuals were:

Search (Fig. 1A): *A. dorsalis* (on the right of the picture) moves across the arena checking the environment with its antennae and palps; as it detects the presence of *B. sclopeta*, it becomes motionless with the antennae stretched forward.

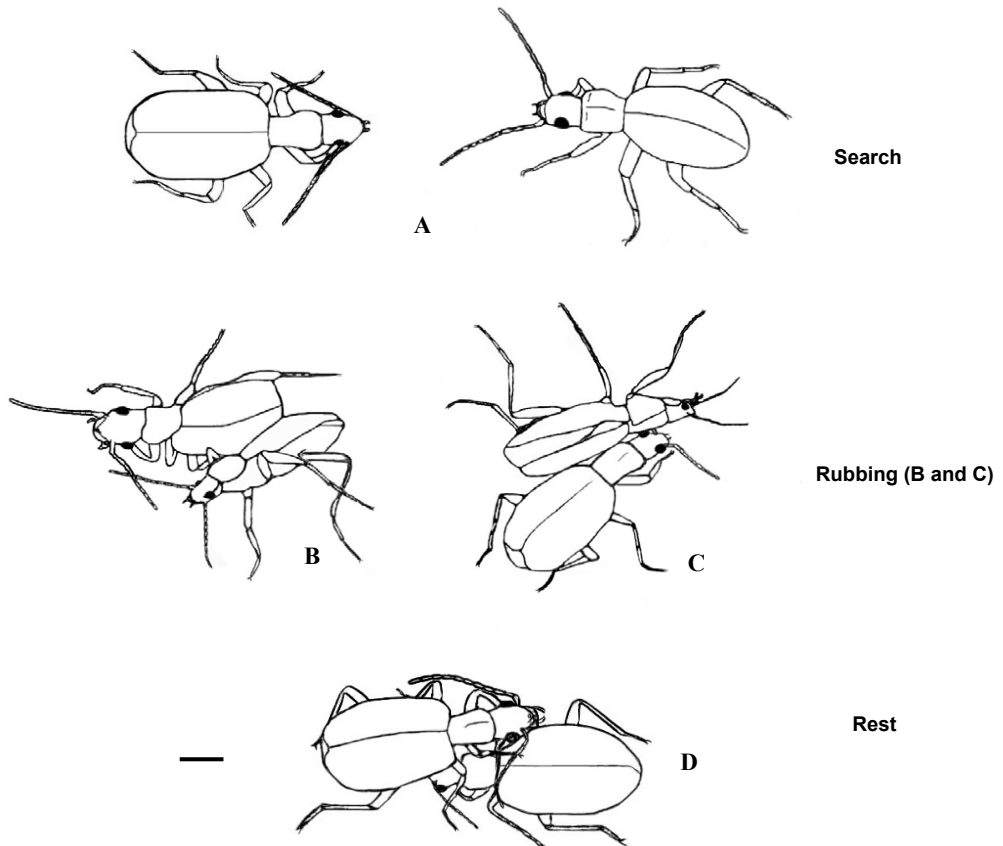


Fig. 1. — Pictures of behavioural events that portray rubbing behaviour in *Brachinus sclopeta* and *Anchomenus dorsalis*. In A *Brachinus sclopeta* is on the left, *Anchomenus dorsalis* on the right. B. *sclopeta* is on the left in B, underneath in C, and on the left; in D. (Scale: 1 mm).

Rubbing (Fig. 1B-C): *A. dorsalis* (on the right in picture A and above in picture B) after detection investigates the body of *B. sclopeta* with both antennae and palps; then, it slides backwards and forwards on its body, rubbing its own head, pronotum and elytrae consecutively. Both the outer antenna and foreleg are rotated backwards during this phase.

Rest: *A. dorsalis* (Fig. 1, on the right of the picture D) stays close to *B. sclopeta*, often under its body; at the same time, it cleans its own abdominal apex and the posterior part of the elytra with its back legs.

The behavioural events displayed by *B. sclopeta* versus *A. dorsalis* were:

Scour: *B. sclopeta* (Fig. 1, on the left of the picture A) walks along the arena border searching for a shelter; it moves slowly, with frequent stops during which its antennae lay backwards at elytra level.

Calm (Fig. 1B-C): *B. sclopeta* rests motionless during the rubbing event, without producing crepitation or showing signs of distress.

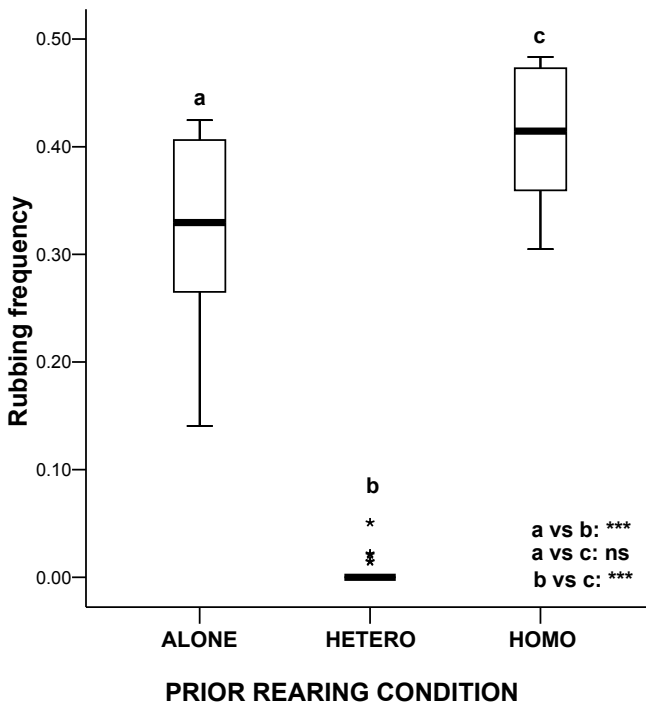


Fig. 2. — Differences in rubbing frequency (express as rubbing event occurrence over the total number of recorded behavioural events) of *A. dorsalis* vs *B. sclopeta* in alone-reared individuals (ALONE), or in animals reared in Hetero- (HETERO) or Monospecific (HOMO) groups. Boxes represent the 1 quartile (25%, lower box extreme), the 2 quartile (median, thick bar), and the 3 quartile (75%, upper box extreme), while whiskers represent the extreme values. Mann-Whitney test results (corrected for multiple comparisons) are reported: \* =  $P_{MonteCarlo} < 0.05$ ; \*\* =  $P_{MonteCarlo} < 0.01$ ; \*\*\* =  $P_{MonteCarlo} < 0.001$ ; NS = not significant.



Moving: during the rubbing event, *B. sclopeta* moves and some times cleans its own back with its legs.

*Comparison of rubbing occurrence*

The occurrence of rubbing behaviour towards *B. sclopeta* significantly differed across the three groups (heterospecific, alone-reared and monospecific groups) ( $\chi^2 = 40.560$ ,  $df = 2$ ,  $P_{MonteCarlo} < 0.001$ ; Fig. 2). In particular, the individuals that were reared in heterospecific groups showed significantly lower frequencies of rubbing than alone-reared ( $U = 0.0$ ,  $W = 253.0$ ,  $P_{MonteCarlo} < 0.001$ ), or test animals reared in monospecific groups ( $U = 0.0$ ,  $W = 253.0$ ,  $P_{MonteCarlo} < 0.001$ ; Fig. 2).

The rubbing behaviour of *A. dorsalis* was more frequent on stationary *B. sclopeta* individuals (rubbing *A. dorsalis* vs still *B. sclopeta*) than when they were moving (rubbing *A. dorsalis* vs moving *B. sclopeta*; Fig. 3), both in alone-reared ( $z = -3.351$ ,  $P_{MonteCarlo} < 0.001$ ) or individuals reared in monospecific groups ( $z = -4.709$ ,  $P_{MonteCarlo} < 0.001$ ). On the other hand no significant difference was detected between

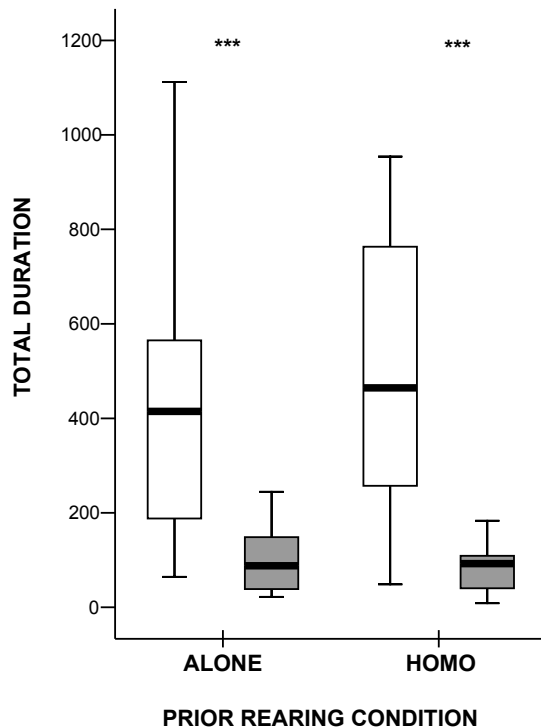


Fig. 3. — Total duration (in sec) of rubbing behaviour of *Anchomenus dorsalis* towards stationary (white boxes) and moving (grey boxes) *Brachinus sclopeta* in alone-reared individuals (ALONE) or those reared in monospecific groups (HOMO). Boxes represent the 1 quartile (25%, lower box extreme), the 2 quartile (median, thick bar), and the 3 quartile (75%, upper box extreme), while whiskers represent the extreme values. Wilcoxon test results (corrected for multiple comparisons) are reported: \* =  $P_{MonteCarlo} < 0.05$ ; \*\* =  $P_{MonteCarlo} < 0.01$ ; \*\*\* =  $P_{MonteCarlo} < 0.001$ ; NS = not significant.

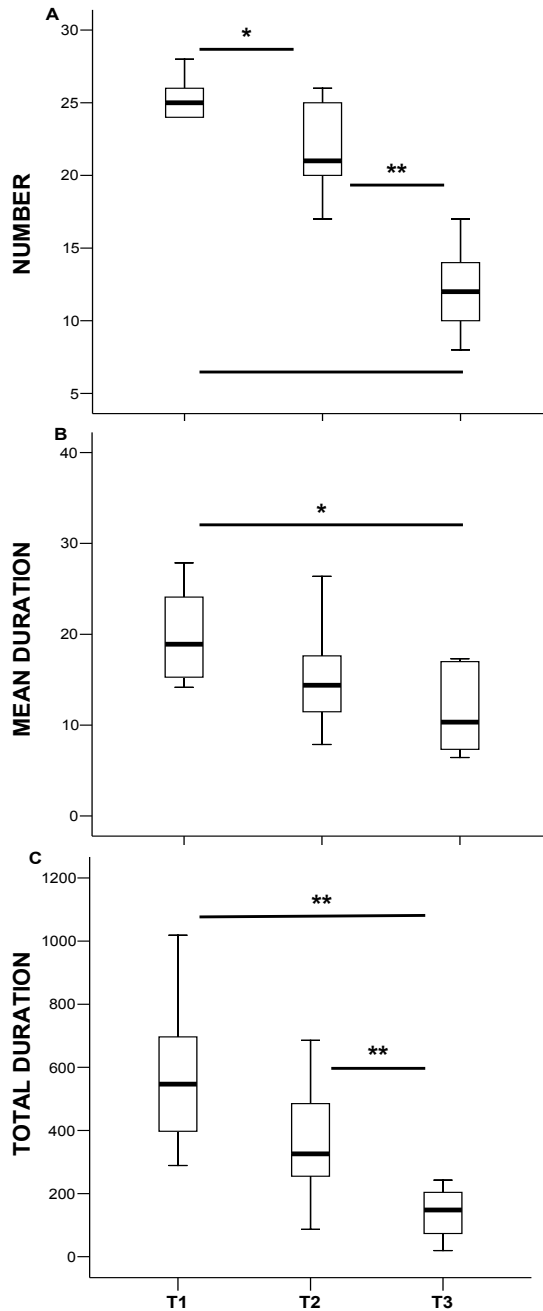


Fig. 4. — Differences in number of rubbing events (A), mean duration of each event (B), and spent rubbing (C). Boxes represent the 1 quartile (25%, lower box extreme), the 2 quartile (median, thick bar), and the 3 quartile (75%, upper box extreme), while whiskers represent the extreme values. Wilcoxon test results (corrected for multiple comparisons) are reported: \* =  $P_{MonteCarlo} < 0.05$ ; \*\* =  $P_{MonteCarlo} < 0.01$ ; \*\*\* =  $P_{MonteCarlo} < 0.001$ ; NS = not significant.

rubbing frequencies on stationary or moving *B. sclopeta*, both for alone-reared *A. dorsalis* as well as for *A. dorsalis* reared in monospecific groups (Mann Whitney tests,  $P_{MonteCarlo} > 0.050$ ).

As far as the rubbing behaviour decrement is concerned (Fig. 4), we observed a significant decrement in the number of rubbing events ( $\chi^2 = 17.897$ ,  $df = 2$ ,  $P_{MonteCarlo} < 0.001$ ), in the mean duration of each event ( $\chi^2 = 12.800$ ,  $df = 2$ ,  $P_{MonteCarlo} < 0.001$ ), and of the total time spent rubbing ( $\chi^2 = 18.200$ ,  $df = 2$ ,  $P_{MonteCarlo} < 0.001$ ).

In particular, the number of rubbing events decreased from T1 to T2 ( $z = -2.492$ ,  $P_{MonteCarlo} = 0.012$ ), as well as from T2 to T3 ( $z = -2.809$ ,  $P_{MonteCarlo} = 0.002$ ). The mean duration of each event did not decrease significantly from T1 to T2 ( $z = -2.090$ ,  $P_{MonteCarlo} = 0.037$ ), nor from T2 to T3 ( $z = -2.293$ ,  $P_{MonteCarlo} = 0.020$ ), but the decrement from T1 to T3 was nonetheless significant ( $z = -2.701$ ,  $P_{MonteCarlo} = 0.004$ ).

Similarly, the total duration of rubbing behaviour in T1 did not differ significantly from T2 ( $z = -2.293$ ,  $P_{MonteCarlo} = 0.02$ ), but differed from T3 ( $z = -2.803$ ,  $P_{MonteCarlo} = 0.002$ ). In this respect, T2 also differed from T3 ( $z = -2.803$ ,  $P_{MonteCarlo} = 0.002$ ).

#### *Rubbing towards a piece of paper (Paper's dummie)*

*A. dorsalis* displayed the rubbing behaviour towards all the pieces of paper previously placed in the monospecific group of *B. sclopeta*, but never towards pieces of paper that were moistened with water.

## DISCUSSION

Until now, behaviours related to inter-specific interactions among carabid beetles have never been described, except for LINDROTH's qualitative description of the rubbing behaviour of *Anchomenus dorsalis* vs *Brachinus* spp. (1949).

Our recordings allowed us both to describe and to quantify the whole behavioural sequence. The behaviour is triggered by the presence of even only one *B. sclopeta* (both male and female). Once the individual *A. dorsalis* perceived the presence of one *B. sclopeta*, it immediately began a "to and fro" movement on the receiver's back (*B. sclopeta*), with head, pronotum and elytra strictly in contact. This behaviour is not mutual, as the receiver is motionless. Indeed, no reaction was recorded in *B. sclopeta*, neither aggressive (crepitation or mandible attack) nor escaping display. The list of behavioural events is likely to be a fixed action pattern with a fixed target, as the behaviour was never generated to no purpose or towards substitute receivers, stones or objects, as occurs with some animals reared in generally good laboratory conditions, but where signals or key *stimuli* are lacking.

Moreover, to our knowledge, although *A. dorsalis* actively interacts with many other aposematic species (*Brachinus* spp., and *Chlaenius* spp.; work in progress), we never observed *A. dorsalis* displaying the rubbing behaviour versus the other 23 ground beetle species that were tested in the present study (see Table 1). This confirms LINDROTH's hypothesis that rubbing may have been considered as a "cleansing ritual". In that case, we would have expected a similar display with no regard to aposematic patterns.

Moreover, our observations revealed that rubbing intensity is higher in animals that have not experienced recent (at least 15 days) interaction with *B. sclopeta*,

both after an isolation period in individual cages, and in monospecific groups. Conversely, "recently experienced" *A. dorsalis* (reared in heterospecific groups) displays the rubbing behaviour with lower intensity and for a shorter time. This differential motivation, and the fact that no significant difference was found in occurrence and duration of rubbing shown by *A. dorsalis* reared alone or in monospecific groups, suggests that rubbing is finalised to satisfy an undefined need, that may force two copulating *A. dorsalis* to interrupt a copulation for rubbing with a *B. sclopeta* individual, indeed (T. BONACCI, personal observation).

Most interestingly, after a frantic initial rubbing, its frequency tends to decline gradually until the rubbing ceases in a couple of hours. This may suggest that the need is satisfied and the goal is reached.

These results, along with the fact that *A. dorsalis* showed the rubbing behaviour towards pieces of paper with *B. sclopeta* odour but not towards other objects or pieces of paper moistened with water, support the hypothesis that the rubbing behaviours involves a transfer or mixing of chemical substances on the cuticle of *B. sclopeta* and *A. dorsalis* individuals.

The adaptive value of this behaviour may be related to the strengthening of an effective antipredatory strategy: as beetles belonging to the genus *Brachinus* are chemically protected, they are easily recognized as unpalatable by experienced predators. In fact, they also present the bright colours typical of aposematic insects, with shining metallic-blue elytrae and a red-orange forebody in *Brachinus*, whereas an inverse colour pattern, with bluegreen forebody and yellow-orange elytrae is found in *A. dorsalis*. Similar patterns occur often in aposematic insects (COTT 1940, GAMBERALE & TULLBERG 1996, GAMBERALE & SILLÈN-TULLBERG 1998, DE COCK & MATHYSEN 2001) and we recently observed that *A. dorsalis* also possesses warning substances to avoid predation by olfactive predators (pyrazines, work in progress).

*A. dorsalis* and *Brachinus* are not closely related phylogenetically within the carabid family, but their populations share more or less the same habitats (R. PIZZOLOTTO unpublished data) and the rubbing behaviour seems to be the core of an interspecific aggregation beneficial for both taxa. Possibly, both species may take advantage from aggregations through a sort of müllerian mimetism (ROTHSCHILD 1961, EISNER & GRANT 1980 in DETTNER & LIEPERT 1994): *B. sclopeta* may increase the dilution effect, while *A. dorsalis* may benefit both from the dilution effect and from the greater chemical defence exerted by *B. sclopeta*.

More work is needed to delineate in detail the evolutionary significance of this behaviour, focusing on the possibility that interspecific aggregations may be not rare in ground beetles, and that this positive interaction could involve a larger bulk of species and genera, at least in wet, sun-exposed and open land habitat types.

## REFERENCES

- ANESHANSLEY D., EISNER T., WIDOM J. & WIDOM B. 1969. Biochemistry at 100°C: Explosive secretory discharge of bombardier beetles (*Brachinus*). *Science* 165: 61-63.
- BALL G.E., CASALE A. & VIGNA TAGLIANTI A. 1998. Phylogeny and classification of Caraboidea (Coleoptera: Adephaga). *Proceeding Symposium XXXX International Congress of Entomology*, Florence, 28 August 1996. *Atti del Museo Regionale Scienze Naturali di Torino*: 1-543.
- BEDÉL L. 1881. Faune des coléoptères du bassin de la Seine. Première partie. *Paris: Société Entomologique de France*.

- BEDEL L. 1895-1914. Catalogne raisonné des Coléoptères du nord de l'Afrique (Maroc, Algérie, Tunisie et Tripolitane) avec notes sur la faune des Îles Canaries set de Madère. I. Paris: *Société Entomologique de France*.
- BERTRAM B.C.R. 1978. Living in groups: predators and prey, pp. 64-96. In: Krebs J.R. & Davies N.B., Edits. Behavioural ecology: an evolutionary approach. Malden, Oxford, Melbourne: *Blackwell Publishing*.
- BONACCI T., MAZZEI A., ZETTO BRANDMAYR T. & BRANDMAYR P. 2004. Aposematic aggregation of carabid beetles (Coleoptera Carabidae): preliminary data. *Redia* LXXXVII: 243-245.
- COTT H.B. 1940. Adaptive coloration in animals. London: *Oxford Press*.
- DE COCK R. & MATTHYSEN E. 2001. Aposematism and bioluminescence: experimental evidence from glow-worm larvae (Coleoptera: Lampyridae). *Evolutionary Ecology* 13: 619-639.
- DETTNER K. & LIEPERT C. 1994. Chemical mimicry and camouflage. *Annual Review of Entomology* 39: 129-154.
- EISNER T. & MEINWALD J. 1966. Defensive secretions of Arthropods. *Science* 153 (3472): 1341-1350.
- EISNER M. 1970. Chemical defense against predation in arthropods. In: Sondheimer E. & Someone J.B., Edits. *Chemical Ecology*. New York: *Academic Press*.
- EISNER T. & DEAN J. 1976. Ploy and counterploy in predator-prey interactions: Orb-weaving spiders versus bombardier beetles. *Proceedings of the National Academy of Sciences of the USA* 73 (4): 1365-1367.
- EISNER T. & GRANT R.P. 1980. Toxicity, odor aversion, and "olfactory aposematism". *Science* 213: 476.
- FINN J.A. & GILLER P.S. 2000. Patch size and colonization patterns: an experimental analysis using north temperate coprophagous dung beetles. *Ecography* 23: 315-327.
- GAMBERALE G. & SILLÈN-TULLBERG B. 1998. Aposematism and gregiousness: the cobined effect of group size and coloration on signal repellence. *Proceedings of the Royal Society of London (B)* 265: 889-894.
- GAMBERALE G. & TULLBERG B.S. 1996. Evidence for a peak-shift in predator generalization among aposematic prey. *Proceedings of the Royal Society of London (B)* 263: 1329-1334.
- GILLER P.S. & DOUBE B.M. 1989. Experimental analysis of inter-and intraspecific competition in dung beetle communities. *The Journal of Animal Ecology* 58: 129-142.
- GOOD P. 2000. Permutation tests: a practical guide to resampling methods for testing hypotheses, 2nd Ed. Heidelberg: *Springer Verlag*.
- HEARD S.B. & REMER L.C. 1997. Clutch-size behaviour and coexistence in ephemeral-patch competition models. *The American Naturalist* 150 (6): 744-770.
- HODEK I. 1973. Biology of the Coccinellidae. Prague: *Academia Press*.
- HUTTON S.A. & GILLER P.S. 2004. Intra- and interspecific aggregation of north temperate dung beetles on standardised and natural dung pads: the influence of spatial scale. *Ecological Entomology* 29: 594-605.
- IVES A.R. 1991. Aggregation and coexistence in a carrion fly community. *Ecological Monographs* 61 (1): 75-94.
- JEANNE C. 1968. Carabiques de la Peninsule Iberique. *Actes de la Société de Linné, Bordeaux* 6 (A): 105.
- JEANNEL R. 1942. Coléoptères Carabique I-II. Faune de France. Paris: *Lechevalier Press*.
- KRIJGER C.L. & SEVENSTER J.G. 2001. Higher species diversity explained by stronger spatial aggregation across six neotropical *Drosophila* communities. *Ecology Letters* 4: 106-115.
- LEE R.E. 1980. Aggregation of lady beetles on the shores of lakes (Coleoptera: Coccinellidae). *The American Midland Naturalist* 104 (2): 295-304.
- LINDROTH C.H. 1949. Die Fennoskandischen Carabidae. *Göteborgs Kungliga Vetenskapsoch Vitterhetø-Samhälles Handlingar, Stockholm (B4)* 1, Spezieller Teil, 1-709 (1945); 3, Allgemeiner Teil. 1-911.
- MADDISON D.R., BAKER M.D. & OBER K.A. 1999. Phylogeny of carabid beetles as inferred from 18S ribosomal DNA (Coleoptera: Carabidae). *Systematic Entomology* 24: 103-138.
- MAGISTRETTI M. 1965. Fauna d'Italia. Coleoptera Cicindelidae, Carabidae. Catalogo topografico. Bologna: *Calderini Press*.

- MAZZEI A., BONACCI T., ZETTO BRANDMAYR T. & BRANDMAYR P. 2005. Capacità di aggregazione di Coleotteri Geodefagi, in ambiente ipolitico di suoli argillosi del bioclima mediterraneo arido. *Atti del XV Congresso della Società Italiana di Ecologia*. Torino, 12-14 settembre 2005.
- MEHTA C.R. & PATEL N.R. 1996. *SPSS Exact Tests 7.0 for Windows®*. Chicago, USA: SPSS Inc.
- MÜLLER G. 1926. I Coleotteri della Venezia Giulia I. Adephaga. *Studi Entomologici. Museo Civico di Storia Naturale di Trieste* 1 (2).
- NIASSY A., TORTO B., NJAGI P.G.N., HASSANALI A., OBENG-OFORI D. & AYERTEY J.N. 1999. Intra- and interspecific aggregation responses of *Locusta migratoria migratorioides* and *Schistocerca gregaria* and a comparison of their pheromone. *Journal of Chemical Ecology* 25 (5): 1029-1042.
- NOVOA F. 1975. Los carabidae de la Sierra de Guadarrama I. Inventario de especies y biogeografía. *Actas de la Real Sociedad Española de Historia Natural* 73: 99-147.
- OBER K.A. 2002. Phylogenetic relationships of the carabid subfamily Harpalinae (Coleoptera) based on molecular sequence data. *Molecular Phylogenetics and Evolution* 24: 228-248.
- RATCHFORD S.G & EGGLESTON D.B. 2000. Temporal shift in the presence of a chemical cue contributes to a diel shift in sociality. *Animal Behaviour* 59: 793-799.
- ROTHSCHILD M. 1961. Defensive odours and mullerian mimicry among insects. *Transactions of the Royal Entomological Society of London* 113: 101-121.
- SCHILDKNECHT H. 1970. Die Wehrchemievon Land- und Wasserkäfern. *Angewandte Chemie* 1: 17-25.
- SCHILDKNECHT H. 1975. Experimente zur Explosionschemie der Bombardierkäfer. *Chemie und Experimente Didaktikchemie* 1: 43-44.
- SEVENSTER J.G. 1996. Aggregation and coexistence. I. Theory and analysis. *The Journal of Animal Ecology* 65: 297-307.
- SEVENSTER J.G. & VAN ALPHEN J.J.M. 1996. Aggregation and coexistence. II. A neotropical *Drosophila* community. *The Journal of Animal Ecology* 65 (3): 308-324.
- SIEGEL S. & CASTELLAN N.J.J. 1988. Nonparametric statistics for the behavioral sciences. *New York: McGraw-Hill Press*.
- SILLÉN-TULLBERG B. & LEIMAR O. 1988. The evolution of gregariousness in distasteful insects as a defense against predators. *The American Naturalist* 132 (5): 723-734.
- SOKAL R.R. & ROHLF F.J. 1995. *Biometry. The principles and practice of statistics in biological research*, 3rd Ed. *New York, USA: WH Freeman & C.*
- THIELE H.U. 1977. Carabid beetles in their environments. A study on habitat selection by adaptations in physiology and behaviour. *Berlin, Heidelberg, New York: Springer-Verlag*.
- WAUTIER V. 1970. Existence d'une interaction olfactive chez Brachinides (Col. Carabidae). *Annales de la Société Entomologique de France* 6 (2): 433-436.
- WAUTIER V. 1971. Un phénomène social chez Coléoptères: le gregarisme de *Brachinus* (Caraboidea, Brachinidae). *Insectes Sociaux* 18: 1-84.
- WOODCOCK A.A., WATT A.D. & LEATHER A.R. 2002. Aggregation, habitat quality, and coexistence: a case study on carrion fly communities in slug cadavers. *The Journal of Animal Ecology* 71: 131-140.
- ZABALLOS P.J.M. 1985. Paralelismo fenológico en *Brachinus variventris* Schauffss, 1862 y *Anchomenus dorsalis* (Pontoppidan, 1763) (Coleoptera Carabidae). *Boletim de la Sociedade Portuguesa de Entomologia* 3 (1): 85-92.