

Predator feeding choice on conspicuous and non-conspicuous carabid beetles: first results

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Abstract

Insects use various types of behaviour, chemical defences, mimetic, aposematic or cryptic appearances as anti-predatory strategies. Among insects, carabid beetles of the genus *Brachinus* are distasteful prey because they discharge an irritating “cloud” of quinones when threatened. These beetles live in aggregations and adopt warning (conspicuous pattern) colours and chemicals to create a template that is easily learnt by predators. Another carabid beetle, *Anchomenus dorsalis*, mimics the colours and cuticular profile of *Brachinus* and is usually found in *Brachinus* aggregations. In this paper we report results from laboratory observations on feeding choice of the following natural predators - *Crocidura leucodon* (Insectivora: Soricidae), *Ocytus olens* (Coleoptera: Staphylinidae) and *Podarcis sicula* (Reptilia: Lacertidae) - on carabid beetle species. Comparing the number of attacks of predators towards aposematic and non-aposematic prey, there was a statistically significant preference towards non-aposematic prey.

Keywords

Brachinus, *Anchomenus*, anti-predatory strategies, warning signals, Coleoptera, Carabidae, laboratory tests

Introduction

Visual and chemical anti-predatory strategies influence trophic webs, as defensive substances (such as semiochemicals or ecomones) (sensu Pasteels 1977, 1982) play an important role (Pasteels et al. 1983) as deterrents against predators. A considerable

amount of work has been done in evaluating anti-predatory strategies and in the identification of defence compounds in arthropods (Eisner 1970; Edmunds 1974; Guilford 1990; Alatalo and Mappes 1996; Gamberale and Tullberg 1998). Many animals use warning colours (or aposematism) to signal their unpalatability to potential predators (Cott 1940; Guilford 1990). In insects, aposematic colouration often co-occur with gregariousness (Edmunds 1974) increasing the effect of the aposematic signal (Poulton 1890; Cott 1940; Rowe and Guilford 1999; Riipi et al. 2001).

In Europe, *Anchomenus dorsalis* (Pontoppidan 1763), which produces methylsalicylate from its pygidial gland (Schildknecht 1970) as well as other chemicals (Bonacci et al., work in progress), is often found with species of the bombardier beetle genus *Brachinus* Weber, 1801 (Wautier 1971; Juliano 1985; Zaballos 1985; Bonacci et al. 2004a; Mazzei et al. 2005; Zetto Brandmayr et al. 2006) and, like *Brachinus*, is brightly coloured (green-blue and red-brown). In terms of chemical defence, bombardier beetles are amongst the best protected insect taxa. When attacked, these beetles eject jets of fluid (with a loud popping sound) from a pair of gland openings on the tip of the abdomen, aiming their discharge with accuracy towards the threat. The active compounds of the secretion are 1,4-benzoquinones, p-benzoquinone and 2-methyl-p-benzoquinone, which are mixed explosively at the moment of ejection, and discharge at 100°C with an audible detonation (Schildknecht 1961; Aneshansley et al. 1969; Eisner 1970; Eisner and Aneshansley 1999; Eisner et al. 2005; Bonacci et al. 2008). A number of predators have been shown to be repelled by bombardier beetles, including ants, carabid beetles, praying mantids, spiders, frogs and toads (Eisner 1958, 2003; Eisner and Dean 1976; Thiele 1977; Dean 1980a, b; Bonacci et al. 2004a, b, 2006).

In this study we report results from laboratory observations on the number of attacks of natural insect predators: *Crocidura leucodon* (Hermann, 1780) (Insectivora: Soricidae), *Ocypus olens* (Müller, 1764), (Coleoptera: Staphylinidae) and *Podarcis sicula* Rafinesque, 1810 (Reptilia: Lacertidae) towards some species of carabid beetles.

Material and methods

The lizard *Podarcis sicula*

Eleven hand collected adult male lizards (*Podarcis sicula*) were used in this study (collected from Cosenza province, southern Italy). Lizards were kept in the laboratory under natural daylight conditions. They were maintained in plastic cages (55 cm length × 34 cm width × 33 cm height) with opaque sides. Prey used were four species of carabid beetles, two of which were conspicuous: *Brachinus sclopeta* (Fabricius, 1792) (N = 11), *Anchomenus dorsalis* (N = 11); and two non-conspicuous: *Amara anthobia* A. Villa & G. B. Villa, 1833 (N = 11), *Amara aenea* (De Geer, 1774) (N = 11). The carabid beetles were collected by hand in the Crati Valley, Cosenza province, southern Italy.

Lizards were tested individually in an open arena (size: 28 cm length × 18 cm width × 16 cm height) with a lamp on a white plaster substrate. During the experiment temperature was maintained at 24–26°C. The trials were performed from June to July 2006. Each lizard was tested once by offering one individual of four prey species (*B. sclopeta*, *A. dorsalis*, *A. aenea*, *A. anthobia*) at the same time. Each carabid beetle was tested once. Before the beginning of the trial, each lizard was not fed for two days. The lizard to be tested was kept in the arena for 10 minutes before starting the trial. The trial began when the four prey individuals was put into the arena and lasted when the prey was ingested. If no predation occurred, the trial lasted for 30 minutes after the prey was put into the arena.

The behaviour of each lizard during the trial was recorded using a digital camcorder (Sony HDV 1080i). Attack delay and whether the carabid beetles were killed or refused were also recorded. Differences between the occurrences of attacking the different prey species were evaluated using the Chi-square test. Attack delay was evaluated using Mann-Whitney and Kruskal-Wallis tests, using the SPSS v.12.0 statistical package.

The staphylinid beetle *Ocypus olens*

Ten adult male staphylinid beetles, *Ocypus olens*, were collected by hand in the field (Cosenza Province, Italy). Each beetle was kept in the laboratory in a climate chamber at 18–24°C under L/D: 18/6 photoperiod. Each individual was maintained in a plexiglas container (10×8×6 cm) with 2 cm of clayey soil. The trials were performed between September 2003 and July 2004. Each beetle was collected four days before the experiment and maintained until the end of the experiment.

The beetles were not fed the day before the trial. Each beetle was individually tested in the laboratory. During each trial, one staphylinid beetle was placed in an arena (10×8×6 cm), followed immediately by adding one of eight carabid prey species (see below). The observation period started immediately and lasted for 10 minutes (for a total of 80 minutes per staphylinid specimen) without a rest period between the interactions.

The order in which the different carabid beetle prey species were introduced to the arena was random. The trials were video-recorded with a Panasonic digital video-camera. We counted the number of attacks towards the different prey species. The model prey consisted of eight species of carabid beetles. Three species possess warning colours and chemical defences (*Brachinus sclopeta*, *Anchomenus dorsalis* and *Chlaenius velutinus* (Duftschmid, 1812)) and five are without these characteristics (*Steropus melas* (Creutzer, 1799), *Calathus fuscipes* (Goeze, 1777), *Pseudophonus rufipes* (De Geer, 1774), *Poecilus cupreus* (Linné, 1758), and *Amara anthobia*). Attack frequency differences between species that possess warning colours and chemical defences, and those who do not possess these characteristics were evaluated using the Chi-square test in SPSS v.12.0.

The shrew *Crocidura leucodon*

Two adult specimens of the shrew, *Crocidura leucodon* (1 male and 1 female), were collected by long worth traps (Pollino mountain, Calabria, 1200 m a.s.l.) in October 2002. The shrews were kept under laboratory conditions in plastic cages (55 cm length × 34 cm width × 33 cm height) with opaque sides under natural daylight conditions. Nine carabid species were used as prey; *Scybalicus oblongiusculus* (Dejeani, 1829), *Parophonus hispanus* (Rambur, 1838), *Steropus melas* and *Calathus montivagus* Dejeani, 1831 (without warning colours and chemical defences) and *Chlaenius chrysocephalus* (Rossi, 1790), *Anchomenus dorsalis*, *Brachinus brevicollis* (= *peregrinus*) (Apfelbeck 1904), *B. sclopeta* and *B. crepitans* (Linné, 1758) (with warning colours and chemical defences). Shrew were tested individually in an open arena (size: 25 cm length × 15 cm width × 18 cm height) with plaster as a substrate and with low-light. Before the start of the trial, each shrew was starved for two days. The order in which the different carabid beetle prey species were introduced into the arena was random. The trials were video-recorded and the number of attacks towards the prey species was evaluated using the Chi-square test.

Carabid beetle nomenclature follows Vigna Taglianti (1993).

Results

We found a statistically significant preference towards non-conspicuous prey by the lizard *Podarcis sicula*. *Amara anthobia* and *A. aenea* were attacked with high frequency (Fig. 1a), while *Brachinus sclopeta* and *Anchomenus dorsalis* with low frequency ($X^2 = 23.76$, DF = 3, $P < 0.001$). Non-conspicuous prey were captured and eaten without difficulty, but when *Brachinus sclopeta* or *Anchomenus dorsalis* were captured, lizards always tossed their heads and then rubbed their snouts on the soil. This is most likely because of the unpalatability of aposematic prey (Bonacci et al. 2008; Bonacci et al., work in progress).

The staphylinid beetle *Ocypus olens* reacted differently to chemically protected and unprotected carabids. Aposematic and chemically protected species (*Brachinus sclopeta*, *Anchomenus dorsalis* and *Chlaenius velutinus*) were attacked with lower frequency ($X^2 = 23.56$, DF = 1, $P < 0.001$) than species without these characteristics (*Poecilus cupreus*, *Pseudophonus rufipes*, *Calathus fuscipes*, *Steropus melas* and *Amara anthobia*). Larger carabid species (*C. velutinus* and *S. melas*) were attacked quicker than smaller-sized species (Fig. 1b) (Bonacci et al. 2006).

The shrew *Crocidura leucodon* attacked and consumed all non-conspicuous and unprotected species of carabids, such as *Scybalicus oblongiusculus*, *Parophonus hispanus*, *Steropus melas* and *Calathus montivagus* (Fig. 1c). *Chlaenius chrysocephalus*, *Brachinus peregrinus*, *B. crepitans*, *B. sclopeta* and *Anchomenus dorsalis* were attacked infrequently ($X^2 = 35.25$, DF = 1, $P < 0.001$) and with difficulty (Fig. 2) (Bonacci et al. 2004b).

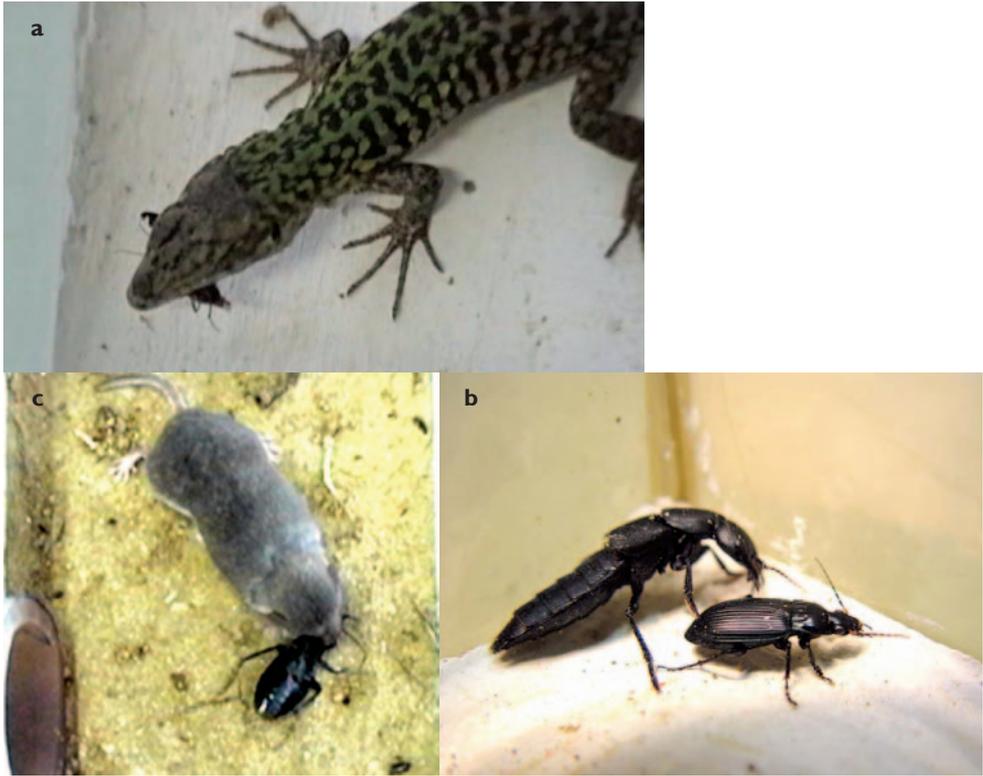


Figure 1 **a** Consumption of *Amara anthobia* by the lizard *Podarcis sicula* **b** attack on *Calathus fuscipes* by the staphylinid *Ocyopus olens* **c** consumption of *Campalita maderae* by the shrew *Crocidura leucodon*.

Discussion

Our results support the hypothesis that conspicuous colouration and defence chemicals in gregarious carabid beetles can produce a sufficient aposematic signal to limit the attack by ambush and active predators. We found a statistically significant preference of predators for non-aposematic prey. Animals protected by chemical defence are often conspicuously coloured (Alcock 1979), since unpalatability is frequently coupled with warning signals (aposematic colours and odours) (Cott 1940; Tullberg et al. 2000). As such, edible prey may exploit the aversion of predators to warning-coloured species and evolve to resemble the model (Joron and Mallet 1998). Moreover, it is likely that unpalatability selects for gregariousness (Alatalo and Mappes 1996). Carabid beetles belonging to *A. dorsalis* use warning colouration and an odour pattern similar to that of *Brachinus sclopeta* (Bonacci et al. 2008; Bonacci et al. work in prep.) to trigger aversion in predators. In Müllerian mimicry, similarity does not necessarily need to be complete (Huheey 1988; Ihalainen et al. 2007), as in the case of *Anchomenus dorsalis* and *Brachinus sclopeta* (Fig. 3), which are quite similar in body size and colour pattern and live in conspicuous aggregations. These results suggest that colouration and chemi-

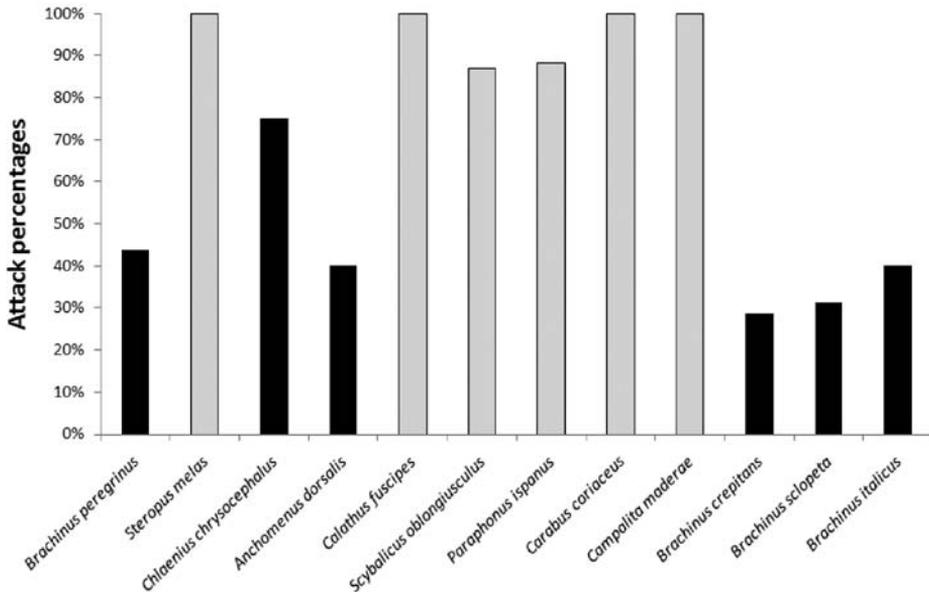


Figure 2. Percentage of attacks by *Crocidura leucodon* (Insectivora: Soricidae) on conspicuous and non-conspicuous carabid beetles. Black bars represent conspicuous species; grey bars represent non-conspicuous species.

icals (multimodal signals) used by the gregarious carabid beetles *Brachinus* spp. and *A. dorsalis* are an efficient anti-predatory strategy. In this case the quinones excreted by *Brachinus sclopetta* and other *Brachinus* species and the methylsalicylate (and probably other warning chemicals) produced by *Anchomenus dorsalis* can act as predator repellents. All predators tested here showed aversion towards *Brachinus* spp. and *A. dorsalis* individuals compared to non-conspicuous species (*Poecilus cupreus*, *Pseudophonus rufipes*, *Calathus fuscipes*, *Calathus montivagus*, *Steropus melas*, *Amara anthobia*, *A. aenea*, *Scybolicus oblongiusculus*, *Parophonus hispanus*).

As suggested by many authors, Müllerian mimicry may influence the diversity of defensive secretions of a species (Rettenmeyer 1970; Edmunds 1974; Pasteels et al. 1983) and in this case, *A. dorsalis* benefits from the different defence systems of *Brachinus* individuals. A similar anti-predatory system has been reported in several reviews concerning insect defence chemistry (Brower 1969; Blum 1981; Nishida 2002); also, Müllerian mimics are sympatric aposematic species that share the same or similar warning patterns (Wickler 1968). The anti-predatory strategies of *Brachinus* spp. and *A. dorsalis* appear to be supported by a combination of conspicuous colouration, defence chemicals and a gregarious habit.

Future chemical and behavioural work should attempt to determine whether species of conspicuous and chemical defence systems are recognizable by the constant emission of odours or by the emission of chemicals after contact with predators (Bonacci et al. work in progress).



Figure 3. Interspecific aggregation of *Brachinus sclopeta* **a** *Anchomenus dorsalis* **b** and individuals of *Pocillus cupreus* **c**. Scale bar = 2 mm.

References

- Alatalo RV, Mappes J (1996) Tracking the evolution of warning signals. *Nature* 382: 708–710. doi: 10.1038/382708a0
- Alcock J (1979) *Animal Behavior. An evolutionary approach*. Sixth Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, 530 pp.
- Aneshansley DJ, Eisner TJ, Widom M, Widom B (1969) Biochemistry at 100°C: explosive secretory discharge of bombardier beetles (*Brachinus*). *Science* 165: 61–63. doi: 10.1126/science.165.3888.61
- Blum M (1981) Chemical defences I. Butterflies. In Vane-Write RI, Ackery PR (Eds) *The biology of butterflies*. London Academic Press, London, 109–134.
- Bonacci T, Mazzei A, Zetto T, Brandmayr P (2004a) Aposematic aggregation of carabid beetles (Coleoptera: Carabidae): preliminary data. *Redia* LXXXVII: 243–245.
- Bonacci T, Aloise G, Brandmayr P, Cagnin M, Zetto Brandmayr T (2004b) Risposte comportamentali di *Crocidura leucodon* (Hermann, 1780) (Insectivora, Soricidae) ai meccanismi antipredatori di alcuni artropodi. *Hystrix It. J. Mamm. (n.s.)* 15: 73–76.
- Bonacci, T, Massolo AG, Brandmayr, P, Zetto, T (2006) Predatory behaviour on ground beetles (Coleoptera: Carabidae) by *Ocypus olens* (Müller) (Coleoptera: Staphylinidae) under laboratory conditions. *Entomological News* 117: 545–551. doi: 10.3157/0013-872X(2006)117[545:PBOGBC]2.0.CO;2

- Bonacci T, Brandmayr P, Dalpozzo R, De Nino A, Massolo A, Tagarelli A, Zetto Brandmayr T (2008) Odour and colour similarity in two species of gregarious Carabid beetles (Coleoptera) from the Crati valley, southern Italy: a case of Müllerian mimicry? *Entomological News* 119: 325–337. doi: 10.3157/0013-872X-119.4.325
- Brower LP (1969) Ecological chemistry. *Scientific American* (New York) 220: 22–29.
- Cott HB (1940) Adaptive coloration in animals. Oxford Press, London, 508 pp.
- Dean J (1980a) Encounters between bombardier beetles and two species of toads (*Bufo americanus*, *Bufo marinus*): speed of prey capture does not determine success. *J Comp Physiol* 135: 41–50. doi: 10.1007/BF00660180
- Dean J (1980b) Effect of thermal and chemical components of bombardier beetle chemical defense: glossopharyngeal response in two species of toads (*Bufo americanus*, *Bufo marinus*). *J Comp Physiol* 135: 51–59. doi: 10.1007/BF00660181
- Edmunds M (1974) Defence in animals: A survey of anti-predator defences. Longman, Harlow, Essex, 357 pp.
- Eisner T (1958) The protective role of the spray mechanism of the bombardier beetle, *Brachynus ballistarius* Lec. *J Ins Physiol* 2: 215–220. doi: 10.1016/0022-1910(58)90006-4
- Eisner T (1970) Chemical defense against predation in arthropods. In: Sondheimer E, Someone JB (Eds) *Chemical Ecology*. Academic Press, New York, NY, 157–217.
- Eisner T (2003) *For Love of Insects*. USA, MA-Cambridge: Harvard Univ Press, 448 pp.
- Eisner T, Aneshansley D (1999) Spray aiming in the bombardier beetle: photographic evidence. *Proc. Natl. Acad. Sci.*, 96: 9705–9709. doi: 10.1073/pnas.96.17.9705
- Eisner T, Dean J (1976) Ploy and counterploy in predator-prey interactions: orb-weaving spiders versus bombardier beetles. *Proc Natl Acad Sci USA* 73: 1365–1367 doi: 10.1073/pnas.73.4.1365
- Eisner T, Eisner M, Aneshansley D (2005) Pre-ingestive treatment of bombardier beetles by jays: food preparation by “anting” and “sand-wiping”. *Chemoecology* 15: 227–233. doi: 10.1007/s00049-005-0316-6
- Gamberale G, Tullberg BS (1998) Aposematism and gregariousness: the combined effect of group size and coloration on signal repellence. *Proceeding of the Royal Society of London* 265: 889–894. doi: 10.1098/rspb.1998.0374
- Guilford T (1990) The evolution of aposematism. In: Evans DL, Schmidt JO (Eds) *Insect defenses: adaptive mechanisms and strategies of prey and predators*. State University of New York Press. Albany, New York, 23–61.
- Huheey JE (1988) Mathematical models of mimicry. *The American Naturalist* 131: 22–41. doi:10.1086/284765
- Ihalainen E, Lindström L, Mappes J (2007) Investigating Müllerian mimicry: predator learning and variation in prey defences. *Journal of Evolutionary Biology* 20: 780–791. doi: 10.1111/j.1420-9101.2006.01234.x
- Joron M, Mallet JLB (1998) Diversity in mimicry: paradox or paradigm? *Trends in Ecology and Evolution* 13: 461–466. doi:10.1016/S0169-5347(98)01483-9
- Juliano SA (1985) Habitat associations, resources, and predators of an assemblage of *Brachynus* (Coleoptera: Carabidae) from southeastern Arizona. *Canadian Journal of Zoology* 63: 1683–1691. doi:10.1139/z85-250

- Mazzei A, Bonacci T, Zetto Brandmayr T, Brandmayr P (2005) Capacità di aggregazione di Coleotteri Geoadefagi, in ambiente ipolitico di suoli argillosi del bioclina mediterraneo arido. Atti del XV Congresso della Società Italiana di Ecologia. Torino, 12–14 Settembre 2005.
- Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. *A. Rev. Entomol.* 47: 57–92. doi:10.1146/annurev.ento.47.091201.145121
- Pasteels JM (1977) Evolutionary aspects in chemical ecology and chemical communication. *Proc. 15th Int. Congr. Entomol.* Washington, 281–293.
- Pasteels JM (1982) Is kairomone a valid and useful term? *J. Chem. Ecol.*: 1079–1081. doi: 10.1007/BF00987889
- Pasteels JM, Grégoire JC, Rowell-Rahier M (1983) The chemical ecology of defense in arthropods. *Ann. Rev. Entomol.* 28: 263–289. doi: 10.1146/annurev.en.28.010183.001403
- Poulton EB (1890). *The colours of animals: their meaning and use especially considered in the case of insects.* Keegan Paul, Trench, Trübner, London, 360 pp.
- Rettenmeyer CW (1970) Insect mimicry. *Ann. Rev. Entomol.* 15: 43–74. doi: 10.1146/annurev.en.15.010170.000355
- Riipi M, Alatalo RV, Lindstrom L, Mappes J (2001) Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature* 413: 512–514. doi: 10.1038/35097061
- Rowe C, Guilford T (1999) Novelty effects in a multimodal warning signal. *Animal Behaviour* 57: 341–346. doi:10.1006/anbe.1998.0974
- Schildknecht HU (1961) Die Bombardierkäfer und ihre Explosionschemie. *Angewandte Chemie* 73: 1–7. doi: 10.1002/ange.19610730102
- Schildknecht HU (1970) Die Wehrchemie von land- und Wasserkäfern. *Angewandte Chemie* 82: 16–25.
- Thiele HU (1977) Carabid Beetles in their environments. A study on habitat Selection by adaptations in physiology and behaviour. Springer Verlag, Berlin, 369 pp.
- Tullberg BS, Leimar O, Gamberale-Stille G (2000) Did aggregation favour the initial evolution of warning coloration? A novel world revisited. *Anim. Behav.* 59: 281–287. doi: 10.1006/anbe.1999.1302
- Vigna Taglianti A (1993) Coleoptera, Archostemata, Adephaga 1 (Carabidae). *In*: Minelli A, Ruffo S, La Posta S (Eds). Checklist delle specie della Fauna Italiana, 44 Calderini, Bologna.
- Wautier V (1971) Un phénomène social chez les Coléoptères: le gréganisme de *Brachinus* (Caraboidea, Brachinidae). *Insect Soc.* 18: 1–84.
- Wickler W (1968) *Mimicry in plants and animals.* London: Weidenfeld and Nicolson, 255 pp.
- Zaballos JP (1985) Paralelismo fenológico en *Brachinus variventris* Schaufuss, 1862 y *Anchomenus dorsalis* (Pontoppidan, 1963). (Coleoptera Carabidae). *Actas do II Congresso Ibérico de Entomologia*: 85–92.
- Zetto Brandmayr T, Bonacci T, Massolo A, Brandmayr P (2006) What is going on between aposematic carabid beetles? The case of *Anchomenus dorsalis* (Pontoppidan 1763) and *Brachinus sclopeta* (Fabricius 1792) (Coleoptera Carabidae). *Ethology Ecology & Evolution* 18: 335–348. doi:10.1080/08927014.2006.9522700