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Carina Härlin

**Phylogeny and evolution of predator
defense in whirligig beetles**

Göteborg University
Faculty of Natural Sciences

**Phylogeny and Evolution of Predator Defense
in Whirligig Beetles**

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Dissertation



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Avhandling för filosofie doktorsexamen i ekologisk zoologi, som enligt Naturvetenskapliga fakultetens beslut kommer att försvaras offentligt fredagen den 4 april 2003, kl. 10.00 i föreläsningssalen, Zoologiska Institutionen, Medicinaregatan 18, Göteborg. Fakultetsopponent är professor Birgitta Tullberg, Zoologiska Institutionen, Stockholms Universitet.

Phylogeny and evolution of predator defense in whirligig beetles

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Abstract

Predation may be seen as a sequence of events: detection, identification, approach, capture and consumption. Arms races between predator and prey have resulted in evolution of a variety of predator-prey systems. This thesis deals experimentally and theoretically with predator defense using whirligig beetles as study organism. Whirligigs live and feed on a variety of freshwater surfaces, often gathering in large aggregations that function mainly in predator defense. From paired pygidial glands the whirligig beetles produce a noxious secretion used in predator defense. The pygidial secretion consists of: a high molecular fraction and a scented volatile fraction. All species produce the high molecular norsesquiterpenes, not all synthesize the volatile fraction (e.g. *Gyrinus minutus* and *G. opacus*) (I). A major theme of this thesis is the role of the volatile secretion in predator defense and signaling (I, II, and III). I show that the volatile secretion functions as an alarm substance in both intra- and inter specific communication. Although only whirligigs producing volatiles are able to use their secretion as an alarm signal, whirligigs lacking volatiles are alerted by volatiles produced by other species (I). Presence of the volatile fraction seems to correlate with group living, habitat choice and predator type (II, III). Lack of volatile secretion seems to make whirligigs more vulnerable to fish predation (II).

Invertebrate predators are often ignored as selective agents in whirligig evolution, however both backswimmers and fish are likely whirligig predators (paper III). In waters containing fish, backswimmers and *G. minutus* are generally concentrated in areas containing emergent vegetation. I show that *G. minutus* (lacking volatiles) survive a backswimmer attack more often than *G. aeratus* (with volatiles) (III). The experimental results suggest that each whirligig species is adapted to defend itself against the predator it is most likely to encounter. Consequently, the pygidial secretion including the volatile fraction is a more effective repellent of fish than of backswimmer. These results suggest that volatile secretions play multiple roles in whirligig anti-predator behavior.

It has also been suggested that whirligig beetles are aposematic using their aggregating behavior as a signal of noxiousness. This is an underlying assumption in this thesis. In the theoretical part, I develop a phylogenetic approach to explain the concept of aposematism stressing its historical nature. Concepts, involving change such as aposematism, are beneficially treated as historical concepts of events. To answer the question *how* aposematism has evolved, we first need to know *where* it has evolved. The *Gyrinus* phylogeny based on absence /presence coding of morphological characters (V) is an important component in explaining whirligig behavior and testing the hypothesis of aposematism.

List of papers

This thesis is a summary based on the following papers, referred to in the text by their roman numerals

- I Borg Karlsson, A-K., Henrikson, B-I., Härlin, C. Ivarsson, P., Stenson, J.A.E. and Svensson B.W. 1999. The possible role of volatile secretion as intra- and inter specific alarm signals in *Gyrinus* species. *Oikos* 87: 220-227.
- II Härlin, C. To have and have not: volatile secretions in gyridid beetles make a difference in predator defense. Manuscript.
- III Härlin, C. Henrikson, B-I., Stenson, J.A.E. and Svensson, J-E. Species-specific predation on gyridid beetles by the backswimmer, *Notonecta glauca*. Manuscript.
- IV Härlin, C. and Härlin, M. 2003. Towards a historization of aposematism. *Evolutionary Ecology* 17: 000-000 (in press).
- V Härlin, C. and Härlin, M. Absent-present coding of morphological characters and the phylogeny of *Gyrinus* (Gyrinidae, Coleoptera). Submitted.

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“Skratt gör att vi tar oss själva mindre högtidligt,
det skapar kontakt med andra och lockar fram vår kreativitet.
Vi blir lättare till sinnet, glädjer oss mer och bekymrar oss mindre”

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“Inne under de lutande bokarna, som sakta fälla sina
gyllene blad över vattenspegeln, just på gränsen mellan
ljus och skugga, ligga några virveldykare. De dåsa i
stillhet och ligga som vilande pärlor på det mörka vattnet,
men närmar man sig blott på några meters avstånd tråda
de genast i den vildaste dans.”

Djurens Värld 2, Insekter I. Malmö 1947.

“Predation is perhaps the most dramatic event in nature, at least from the prey’s point of view” (Svensson 1996).

Introduction

Predation may be seen as a sequence of events: detection, identification, approach, capture and consumption (Vermeij 1982; Endler 1986). A primary defense is a defense reducing the risk of detection and encounter (Edmunds 1974), e.g. crypsis, aposematism and mimicry. Crypsis, when animals bear a resemblance to its natural surroundings, is the most common way to escape predators (Edmunds 1974; Endler 1986). Once discovered, the prey must be identified as edible or not. A way to avoid predators in this phase is to be unpalatable in combination with a warning signal to advertise this unpalatability, i.e. to be aposematic. Another way is to resemble an aposematic prey but lack the unpalatability (batesian mimicry) and avoid being attack due to the incorrect identification to the predators (Endler 1986). In addition the secondary defense works in encounters with predators (Edmunds 1974). For example, an approach by a predator may be hindered by a startle behavior. And last, toughness, spines and noxiousness may prevent a capture and final consumption.

This thesis focuses on predator defense, both experimentally and theoretically using whirligig beetles as the study species. Although whirligig beetles are also known as gyrenid beetles, walzing beetles, scuttle bugs and due to the odor of some species, as, “apple-bugs” and “mellow bugs” (Le Conte 1868, Leech and Chandler 1963), the name whirligig is most apt as it describes the beetle’s whirling and gyrating movement on the water surface. The whirligigs live and feed on a variety of freshwater surfaces, often gathering in large aggregations. These aggregations may function in predator defense in many ways (Vulinec and Miller 1989). For example, they increase the startle and confusion effects to the attacking predator (Newhouse and Aiken 1986). Furthermore, in their paired pygidial glands whirligig beetles produce a noxious, often scenting secretion suggested to be used in several ways as predator defense (e.g. Benfield 1972; Henrikson and Stenson 1993; paper I). It has been suggested that whirligig beetles are aposematic using their aggregating behavior as a signal of noxiousness (Henrikson and Stenson 1993). This hypothesis of aposematism in

whirligig beetles is an underlying assumption in this thesis and something that I discuss from a theoretical and phylogenetic perspective in paper IV. The whirligig phylogeny (paper V) is a necessary component for evaluating the hypothesis of aposematism in whirligig beetles. The whirligig pygidial secretion comprises of a high molecular fraction and a volatile fraction. All species produce the high molecular norsesquiterpenes, but presence of the volatile fraction seems to correlate with group living and habitat choice. The beetles may also use their volatiles as an alarm signal in both intra- and inter specific signaling (paper I). Invertebrate predators have generally been ignored as potential selective agents in whirligig evolution, although both backswimmers and vertebrates are likely whirligig predators of (paper II and III).

Aim of thesis

In this thesis I address the following issues: does it make a difference to have or lack volatiles for whirligigs in their defense against predators? (paper III); can the pygidial secretion produced by one species be received and elicit escape responses in other species even if the receiver lacks volatiles? (paper I); does the production of a high molecular fraction like gyridinal function as the main component in the whirligigs defense against fish predation? (paper III); are backswimmers potential threats to whirligig beetles and, if so, do the pygidial secretion function also to deter invertebrate predators? (paper II); what is aposematism and how should one detect it? (paper IV); and last, I use absence/ presence coding of morphological characters to infer the phylogeny of *Gyrinus* (paper V) for being able to address the issue of aposematism in *Gyrinus*.

The whirligig beetles

Systematics

Gyrinidae is a part of the aquatic adepagan subtaxon Hydradephaga that recent molecular studies suggest to be monophyletic (e.g. Ribera *et al.*, 2002). There is also a consensus that Gyrinidae is the most basal lineage of Adepfaga or Hydradephaga (Beutel and Roughley, 1988; Beutel, 1993, 1995; Beutel and Haas, 1996, 2000; Caterino *et al.*, 2002; Ribera *et al.*, 2002). The family Gyrinidae is the second largest with more than 900 described species (Franciscolo 1979) divided into 13 genera: *Andogyrus*, *Aulonogyrus*, *Dineutes*, *Enhydrus*, *Gyretes*, *Gyrinus*,

Heterogyrus, *Metagyrimus*, *Macrogyrus*, *Orectochilus*, *Orectogyrus*, *Porrorhynchus* and *Spanglerogyrus* (Beutel 1990). Most systematic studies so far have only considered the relationships among the recognized genera of Gyrinidae (e.g. Beutel and Roughley, 1988, 1994; Oygur and Wolfe, 1991; Beutel, 1990, 1993, 1995) with few studies focusing on phylogeny among species within genera (but see Oygur and Wolfe 1991). Gyrinid representatives in most molecular studies boil down to a single species of *Gyrinus* and *Spanglerogyrus albiventris* (e.g. Shull *et al.*, 2001; Caterino *et al.*, 2002). The recent study of Hydradephaga (Ribera *et al.*, 2002) is an exception and includes, besides a *Gyrinus* species and *Spanglerogyrus albiventris*, one representative each of *Aulonogyrus*, *Orectochilus*, *Andogyrus*, *Macrogyrus*, and *Gyretes*. The internal phylogeny of Gyrinidae presented by Ribera *et al.* (2002) is basically congruent with hypotheses based on morphology presented by Beutel and Roughley (1994) with the slight difference that *Macrogyrus* comes out as closer to *Orectochilus* and *Gyretes* rather than sister to *Andogyrus*. Most studies seem to agree that *Aulonogyrus* is the sister taxon to *Gyrinus* (e.g. Beutel and Roughley, 1994; Oygur and Wolfe, 1991; Ribera *et al.*, 2002). The majority of all ecological work is done on *Dineutes* and *Gyrinus*, but in Sweden only *Orectochilus* and *Gyrinus* are represented. *Gyrinus* is the genus of my primary focus and it has a world-wide distribution with some 140 species and in Fennoscandia and Denmark 13 species are known (Holmen 1987).

Paper V I addressed the internal phylogeny of *Gyrinus* (fig xx) as well as making a brief comment on the more inclusive phylogeny within Gyrinidae.

Natural history

Whirligig beetles are adapted to a life in water, with most species inhabiting fresh water sources like: lakes, ponds, swamps, gravel-pits and streams that are not densely vegetated (Svensson 1969). Some species also inhabit brackish water. Most species prefer clean oxygen-rich habitats essential for whirligig larval development (Brinck 1955), but a few species live in polluted water (Holmen 1987). Many whirligig species gather in large aggregations on the water surface, commonly under overhanging tree branches. Adult whirligigs are scavengers feeding on insects trapped on the water surfaces e.g. adult *Diptera*, *Ephemeroptera* and *Trichoptera* (Svensson 1969; Heinrich and Vogt 1980). The size of the whirligig beetles range between 2,5 to 26 mm. (In Fennoscandia and Denmark whirligig beetles range in size from 3 to 8,5

mm, Holmen 1987). The female whirligigs are, like females of many other insects, usually larger than conspecific males. Adult whirligig beetles may live up to three years (Svensson, pers. comm.) and during the winter the beetles lie dormant, hibernating in the mud at the bottom of lakes (Svensson 1985). They come out in spring often when there still are some ices on the water. The whirligig beetles reproduce in May and June (Svensson 1969; Eijk 1986), but late emerging adult females (July and August) are also capable of reproducing in late summer (Eijk 1986). Eggs are laid under water and they adhere to plants, stones, branches or other objects. Development from egg to teneral takes between 6- 10 weeks (Eijk 1986) and of that the incubation time of the eggs varies between 8-12 days (Holmen 1987). The whirligig larvae are predators preying on small bottom dwelling invertebrates like Chironomidae larvae. The whirligig larvae build cocoons from substances like: sand, grains, small fragments of rocks, seeds, flower heads and pieces of woods (Svensson 1985, Holmen 1987; Oygur and Wolfe 1991) in which they pupate. However, the knowledge of the whirligig larvae is still poor, but a few days terrestrial pupal state precedes the adult state (Svensson 1985).

Predator defense

The on going arms race between predator and prey has resulted in the evolution of a variety of anti-predator and efficient predation systems. Crypsis, electricity, spines, odor, bad taste, sounds, behaviors and others, many are the ways prey has evolved to get away being preyed upon (Edmunds 1974).

Whirligig beetles living on the water surfaces are maybe easy discovered by predators. A life on the surface also means whirligigs may be attacked by predators from three sides: above, beneath and from the side. However, not many predators have been shown to feed on them (Benfield 1972; Stenson 1979). Whirligigs have evolved a variety of anti-predator mechanisms. For instance, the whirligigs have divided eyes presumably assimilating information from below and above the water surface (Oygur and Wolfe 1991) and they have a hard waxy elytra providing mechanical protection. They are also very fast swimmers, with speeds up to 1.44 m/s (Vulinec 1987), accomplished by 50 to 60 hits per second with their hind legs (Nachtigall 1965) and are capable of diving or flying should a predator be encountered. Furthermore, many whirligigs live in large aggregations and produce a noxious pygidial secretion, which

possesses several anti predator functions (Benfield 1972; Vulinec and Miller 1989; Henrikson and Stenson 1993).

Aggregation behavior

“Gregariousness is defined as the tendency of an animal to aggregate with others such as that the animals are in contact with one another, or are nearly so...” (Vulinec 1990). The most universal function of lasting aggregations appears to be predator defense (Vulinec 1990). Group living reduces the predation risk by increasing vigilance and each individual in the group may spend less time scanning for predators (Krebs and Davies 1993). Predators may become confused when attacking a dense group of prey items (Neill and Cullen, 1974; Endler 1986). Dilution, where the risk of being preyed upon is lower for an individual in a group than solitary individual, is another overall benefit of group living (Krebs and Davies 1993). Calvert *et al.* (1979) showed that the advantage of dilution outweighed any disadvantage of increased roost conspicuousness. Disadvantages of group living include higher exposure to parasites, competition of resources and groups are more easily discovered (Krebs and Davies 1993). There are however several benefits of aggregating unrelated to anti predation like increased mating success (Heinrich and Vogt 1980) and the access to information pertaining to viable feeding sites (Alcock 1969). There are also physiological functions of insect aggregations, e.g. thermoregulation (Vulinec 1990), but not applicable to the whirligig beetles.

The main function of aggregating in whirligig beetles seems to be anti-predator defense (Benfield 1972; Heinrich and Vogt 1980; Vulinec and Miller 1989; Henrikson and Stenson 1993). Watt and Chapman (1998) suggested whirligig aggregations to behave as selfish herd due to fish attack rate increased with group size, Heinrich and Vogt (1980) claimed that the aggregations formed only in areas where no predators were present or where predators had learnt to avoid whirligigs. Studies have shown that fish only attacked whirligigs dispersing from the aggregations (Heinrich and Vogt 1980). The whirligigs gregarious lifestyle does not seem to be related to increased mating success since whirligigs aggregate all year round, not just during the mating season (Heinrich and Vogt 1980; Vulinec and Miller 1989, Henrikson and Stenson 1993). Additionally several whirligig species commonly co-exist in the same aggregations which cannot increase mating success. However the

above statement that whirligig aggregations exist where there are no predators (Heinrich and Vogt 1980) and preliminary results from a recent study seem to indicate that the aggregations affect both predator defense and are incorporated in mating. Groupings comprising primarily of females and one to two males are more common in springtime than any other part of the year (Härlin unpubl.). The dispersals of the whirligigs among ponds are also common which give populations of whirligigs a frequent gene flow (Nürberger and Harrison 1995). Nevertheless, Vulinec and Miller (1989) showed that individual whirligigs in large groups react faster to predator stimuli than individuals in smaller groups. That is large groups detect predators when they are further away than small groups (Watt and Chapman 1998). Living in aggregations provides enhanced vigilance and thus more eyes to the group as suggested by the "Trafalgar effect" (Treherne and Foster 1980). It has been shown that certain whirligig beetles disperse from the aggregations at night to forage singly (Heinrich and Vogt 1980). However our north European species seem to be more or less active feeding both day and night (Holmen 1987). A study in the southern Sweden also show that whirligigs disperse from the aggregations and move from the open waters to more sheltered areas at dusk when the water bat, *Myotis daubentonii*, starts to forage (Edland and Olsson, in prep.)

Escape reactions

Usually an aggregation of whirligigs is located on the water surface just a few meters from the shore. These whirligigs swim slowly, sometimes in small circles, trying to maintain their position within the aggregation (Freilich 1986, 1989; Romey 1995). According the selfish herd hypothesis the predation risks are higher at the edge of a group. Romey (1995) showed that hungry whirligigs were found closer to the edge of the aggregation and had a higher distance to their nearest neighbor than well-fed beetles. However, when a potential predator's shadow falls on a whirligig, the whirligig starts to swim in rapid haphazard zigzag patterns. This unsystematic motion makes it impossible for the predator to predict the beetle's next position, an example of protean behavior (Newhouse and Aiken 1986). Together with this fright response the alerted beetle produces small hydromechanical water waves which the other whirligigs detect via their Johnston's organ (a mechano receptor in the antennae), prompting them to exhibit the same escape response, the so-called

Trafalgar Effect (Treherne and Foster 1980, Newhouse and Aiken 1986, Vulinec and Miller 1989). With all beetles in the aggregation swimming around in haphazard zigzag motions it is virtually impossible for the predator to follow and capture a beetle. In addition, the whirligig beetles can escape by diving or flying away (Newhouse and Aiken 1986; Henrikson and Stenson 1993). However, if a predator manages to capture a whirligig beetle, the beetle's hard elytra prevents it from being instantly killed allowing the whirligig to release its pygidial secretion which causes the predator to spit it out alive. This is a possible scenario if the predator are a fish, bird, newt or a small mammal (Benfield 1972, Meinwald *et al.* 1972, Miller and Mumma 1976, paper II, but see paper III).

Pygidial secretion (paper I)

A striking synapomorphy (derived character) for the adepagan beetles as a whole is the paired pygidial gland that produces a noxious chemical secretion (Beutel 1995). Since the chemical composition of the aromatic secretion differs between groups it is hypothesized to have evolved separately, once in the group comprising Dytiscidae, Amphizoidae, Noteridae, Haliplidae, and Hygrobiidae; once in Cicindelidae; once in Carabidae; and once in Gyrinidae. Especially Gyrinidae is considered to produce a highly divergent composition of aromatic pygidial gland secretion (Dettner 1987).

The whirligig pygidial secretion is both repellent and toxic to vertebrate predators (Benfield 1972, Meinwald *et al.* 1972; Miller *et al.* 1975; Miller and Mumma 1976). Other proposed functions of the secretion are: to prevention of microorganisms attachment to the whirligig body, to increase the wettability of the chitin cover after either a stay on land or a flight (Dettner 1985), an alarm signal (Henrikson and Stenson 1993, paper I) and action as a propellant (Vulinec 1987). The secretion comprises of two major components, a high molecular fraction and a volatile fraction (Dettner 1979, Ivarsson *et al.* 1996). The high molecular fraction consists of varying amounts of the norsesquiterpenes: Gyrinidal, Isogyrinidal, Gyrinidone and Gyrinidione (Miller *et al.* 1975; Scrimshaw and Keerfoot, 1987)(Fig. 1) while the volatile fraction comprises of compounds such as 3-methyl-1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 6-methyl-5-hepten-2-on (Fig. 2)(Ivarsson *et al.* 1996).

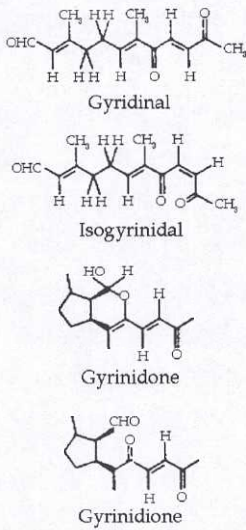


Figure 1. Structure of the four major norsesquiterpenes in the pygidial glands of whirligig beetles (from Schrimshaw and Keerfoot 1987)

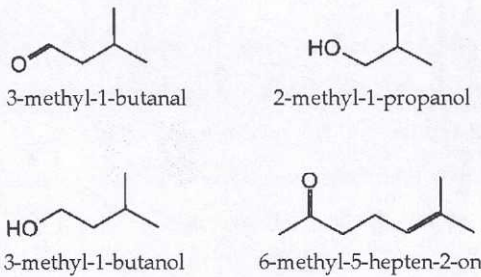


Figure 2. Structures of the volatile compounds in the pygidial secretion of whirligig beetles (from Ivarsson *et al.* 1996).

Volatile compounds give the secretion its characteristic odor and substances in the high molecular fraction were tested as the active agents leading to fish avoiding the whirligig beetles (Miller and Mumma 1976; Eisner and Aneshansley 2000; but see paper II). Additionally, volatiles are suggested to act as alarm substances (Henrikson and Stenson; paper I). Henrikson and Stenson (1993) showed that the strongly scented *Gyrinus aeratus* emitted pygidial secretion which elicit escape responses in other *G. aeratus* individuals. *Gyrinus aeratus* also show a high tendency to aggregate. Thus, they benefit being able to receive alarm signals when threatened. However, the amounts of volatiles produced by different species are highly variable. The scentless *G. opacus* and *G. minutus* produces no or very low amounts volatiles whereas the scenting *G. aeratus* and *G. substriatus* produces at least one order of magnitude more amounts of volatiles (Ivarsson *et al.* 1996; paper I). Though, production of the

norsequiterpenes does not necessarily correlate with a production of the volatile components as *G. minutus* and *G. opacus* also produce the high molecular component gyridal (Schilknecht 1976; paper I).

The species differences in volatile production raised the hypothesis: whether the pygidial secretion emitted from one species can act as an alarm substance eliciting escape responses in other species? (paper I), with the prediction that only species emitting volatiles are able to alert other individuals. In paper (I) we also looked for the similarities and/ or differences in the behavior between smelling and non-smelling species in the field.

The experimental results show that both *G. substriatus* and *G. opacus* react with “fright” responses when exposed to secretion emitted from *G. substriatus*. *Gyrinus opacus* also reacts with escape response to secretion of *G. aeratus*. However, none of the tested species responds to pygidial secretion emitted from *G. opacus*, including *G. opacus* itself. Hence, our prediction was supported by the results and we believe that, if a pygidial secretion is to function as alarm signal it must include the volatile fraction (paper I). Stated more simply, scented species have an alarm signal whereas scentless do not (paper I). Furthermore, the field study showed that scented there are more similarities (e.g. aggregation behavior) between scented whirligig beetles species versus unscented species (Table 1) (paper I).

Others have also pointed out that the evolution of chemical defense and alarm signals seems to be correlated with the habit to form aggregations (e.g. Edmunds 1974, Sillén-Tullberg 1988, Guilford 1990, Gagliardo and Guilford 1993, Krebs and Davies 1993, Gamberale and Tullberg 1998). This hypothesis would be interesting to study further with a comparative and phylogenetic approach in accordance with what is said in paper (IV)(see paper IV for more details). Furthermore, it is not known whether the production of volatile secretion is ancestral in *Gyrinus* and that some species (e.g. *G. minutus* and *G. opacus*) later have lost the ability to produce volatiles or whether the lack of volatiles is the ancestral condition for *Gyrinus* (question raised in paper I, but see also paper IV). The same is also true with respect to aggregating behavior, so “it is evident that well-founded phylogenetic hypotheses are a necessary prerequisite for the development of evolutionary scenarios” (Beutel and Haas 2000, paper IV, V). As a step towards answering these questions we have inferred the phylogeny of *Gyrinus* (paper V).

Table 1. Pygidial secretion fractions and behavioral characteristics of two scented and two unscented gyrinus species. a) presence of high molecular weight secretion (toxic for fish), b) presence of volatile fractions (alarm substances; scented beetles), c) aggregations tendency on exposed water surface, and d) preference solitary or in loose groups) for marginal zones with emergent thin vegetation. (0) refers to a weak although scentless production of volatiles with no alarm function. + refers to a strong aggregation tendency and preference for the marginal zone, respectively; - refers to a weak or no tendency of preference.

	High molecular	Volatile secretion	Aggregation tendency	Preference for emergent marginal vegetation
<i>G. aeratus</i>	+	+	+	-
<i>G. substriatus</i>	+	+	+	-
<i>G. opacus</i>	+	(0)	-	+
<i>G. minutus</i>	+	0	-	+

Predators on Whirligig beetles

As mentioned previously adult whirligig beetles are not noticeably prey upon by predators like frogs, fish, birds, newts and small mammals (Forbes 1888; Wilson 1923; Benfield 1972; Meinwald et.al 1972, Miller and Mumma 1976; Stenson 1979; Newhouse and Aiken 1985; Romey 1995). But, whirligig larvae may be more exposed to predation. However, adult whirligigs have been documented as prey of at least two European bird species, i.e. black tern (*Chlidonias niger*), the greenshank (*Tringa nebularia*) the Wilson's phalarope (*Phalaropus tricolor*) in Canada (Paper I and references therein) and captive Water rats, *Nectomys squamipes*, (Ernest and Mares 1986).

Experiments using fish as potential whirligigs predators show that fish initially taste the whirligigs, but generally expel them and reject them as prey (Benfield 1972; Meinwald et al. 1972; Miller and Mumma 1976; Paper II and III). It has been suggested that substances in the high molecular fraction of the secretion were the

active agents causing the fish rejecting the beetles (Miller and Mumma 1976; Eisner and Aneshansley 2000; but see paper III). However other studies have shown that volatiles are irritants and even toxic to mammals and bacteria (Klimish and Hellwig, 1995; Wright *et al.* 1991).

The above observations prompt the question: Are the norsequiterpenes more effective than the volatiles in whirligig beetles' defense against fish predators? (paper II). Furthermore, nowhere in the literature are invertebrates mentioned as potential whirligig beetles predators, especially since both water striders and backswimmers utilizing the same water surface, as the whirligig beetles (Kolmes 1985). The backswimmer (*Notonecta*) is known to prey on almost any aquatic invertebrate or vertebrate that is smaller or of the same size as itself, (Dolling 1991, Streams 1992, McGavin 1993, Henrikson 1990). Thus, backswimmers may be important whirligigs predators on whirligigs particularly in waters where fish are absent. In paper (III), we address the question: are invertebrate predators a real threat for surface living whirligig beetles?

Finally, it is possible that the secretion have evolved primarily as a defense against bacteria and other microorganism. If so, aggregated whirligigs are probably more protected than to solitary species.

Predation experiments (paper II and III)

In paper II and III we ask the following questions: Are backswimmers potential whirligig predators? If yes, does the pygidial secretion function as a defense? Furthermore, is the norsequiterpenes the main component in the whirligigs chemical defense? To what extent do the backswimmer (i.e. *Notonecta glauca*) and rainbow trout (*Oncorhynchus mykiss*) attack, capture, and ingest whirligig beetles? And, is there a difference in predator behavior when presented with beetles lacking volatiles (e.g. *G. minutus*) or possessing volatiles (e.g. *G. aeratus*)? In paper II, the fish's oral flushing behavior after capturing a whirligig is also studied. By flushing behavior I mean when the fish take the whirligig into its mouth and starts to open and close the mouth in a slow rhythmic action. This behavior is suggested to be a general behavior by fish ridding noxious prey from chemicals (Eisner and Aneshansley 2000).

The prey species *G. minutus* and *G. aeratus* were chosen with respect to differences in defense strategies and habitat choice and size with *G. minutus* (3.0-4.7 mm) smaller than *G. aeratus* (4.4-6.3 mm).

Our hypothesis were that since *G. minutus* mainly live in the same habitats as the backswimmer, *Notonecta glauca*, it has evolved more efficient strategies to escape from the backswimmer than *G. aeratus*. *Gyrinus aeratus* more seldom confront this type of predator in its natural habitat. Conversely, *G. aeratus* who is mainly found in open water areas should have evolved more effective strategies (e.g. volatiles and aggregating behavior) to escape attacks from predators like fish.

The experiments revealed that *N. glauca* is a potential gyrid beetle predator since they captured and consumed whirligigs but also that the whirligigs have minor use of their pygidial secretion against this type of predator with sucking mouth parts. As predicted the backswimmers were also significantly better at capturing the larger odorous species *G. aeratus* than the smaller, scentless, *G. minutus*. The backswimmer successfully captured *G. aeratus* about 50% of the time while it only succeeded 5.6% of the time with *G. minutus* (paper II). However, the rainbow trout's capture efficiency was equal for both whirligig species, but the fish expelled *G. aeratus* more often than *G. minutus* and *G. minutus* was consumed significantly more than *G. aeratus* (Fig. 3 A-D). Hence, *G. minutus* are less distasteful compared to *G. aeratus*. As predicted, *G. aeratus* with production of both norisoprenoids and volatiles are better protected against fish predators than *G. minutus*. That is norisoprenoids are not the only important defense chemical. Furthermore, most rainbow trout exposed the whirligigs to oral flushing immediately after a successful capture (Fig. 3 A-D), but they do not show this flushing behavior when fed palatable crickets. But, the rainbow trout do show disparities in flushing behavior towards the different whirligig species. They spent significantly longer time flushing a *G. minutus* before consuming the beetle than before rejecting it. And when the rainbow trout had experienced each whirligig species respectively, fewer fish flushed *G. aeratus* than *G. minutus* and those flushing *G. aeratus* flushed significantly shorter time than without experience. Consequently, oral flushing may be used only when the fish considers the prey is edible and when they have a chance to rid the item from unpleasant chemicals.

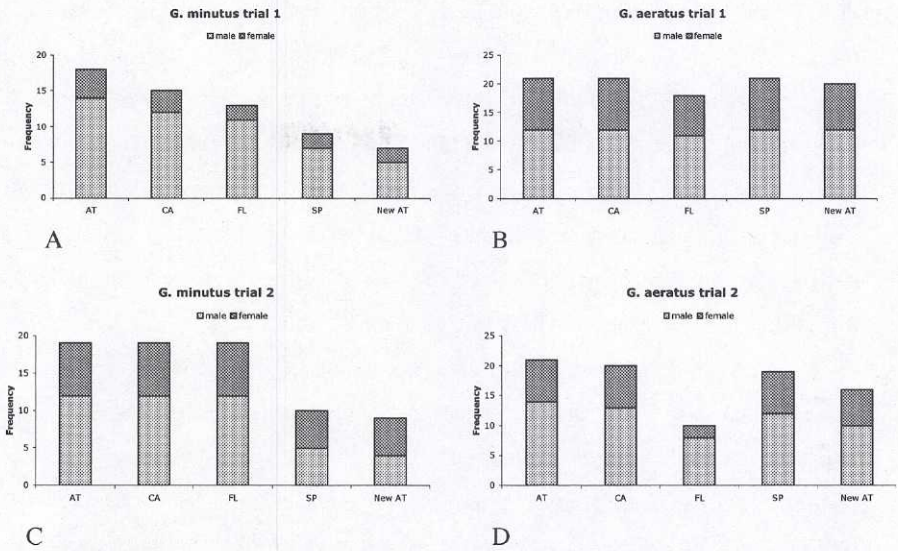


Figure 3. The frequency of fish showing various predator behaviors in a predation sequence. The sequence starts with first attack (AT), first capture (CA), first flushing (FL), first spitting out (SP), and ends with a new attack (New AT). Male-female distributions are also shown. First trial with *G. minutus* (A), first trial with *G. aeratus* (B), second trial with *G. minutus* (C), and second trial with *G. aeratus* (D).

The experimental results from papers II and III show that *G. aeratus* is better defended against fish whereas *G. minutus* is better defended against backswimmers, i.e. each whirligig species are best defended against the kind of predator they are more likely to encounter. Furthermore, the pygidial secretion including the volatile fraction is working well against fish predation, but seems to be less effective as a repellent to backswimmers. Backswimmers have piercing mouthparts and suck the body fluid from their prey and, consequently, they may be able to avoid the whirligigs' emitted pygidial secretion, whereas the fish take the whole beetle into its mouth. Probably it is the alcohols giving rise to the bad taste of the whirligigs (Borg Karlsson pers. com.). The gills of the fish may more easily take up the alcohols than the norsesquiterpenes, or the alcohols may facilitate penetration of the defense compounds (norsesquiterpenes) into predator circulation systems. It is also possible that fish remember the scenting volatiles more easily than the norsesquiterpenes, i.e. it is likely

that they taste differently. Experiments have shown that quail avoidance of ladybirds was sustained by a combination of smell, taste and color pattern (Marples *et al.* 1994) with ladybird odor on its own incapable of maintain a strong control over the behavior (Marples and Roper 1996). Similarly in this study the fish appeared indifferent to volatile odors as they attacked a new whirligig with the same degree of vigor following exposure to volatiles. Instead it seems to be the volatile taste that is the more effective defense mechanism, because not until the fish have the beetles in their mouth there are differences between those that have and have not volatiles.

As showed in paper I, both *G. aeratus* and *G. substriatus* gather in aggregations while *G. minutus* and *G. opacus* live more solitarily. It has been hypothesized that aggregation formations by distasteful species may results in pooling of defensive compounds (Vulinec 1990) to enhance the defense against bacteria and microorganisms as well as to predators. In addition, the aggregations may serve as a warning signal of the whirligig's noxious secretion (Henrikson and Stenson 1993). Grouping may enhance the effect of aposematic prey's chemical or morphological defense, (Sillen-Tullberg 1990 and references therein). Also "the signal effect *per se* (of either colour or behaviour), may be enhanced and give rise to better avoidance [in grouping animals]" (Sillén-Tullberg 1990; see also Gamberale and Tullberg 1996) Gagliardo and Guilford (1993) showed that aggregations enhance the effectiveness of the visual, not the chemical defense. Predators also learn faster to avoid aggregated rather than solitary aposematic prey (Gagliardo and Guilford 1993; Gamberale and Tullberg 1998). Brönmark *et al.* (1984) also showed that trout learned to avoid grouped prey faster than ungrouped.

The case for aposematism in whirligig beetles

The fascination of animal defenses such as aposematism and Batesian mimicry has a long tradition in biology, but the evolution of aposematism is still a puzzle to evolutionary biologists. How could conspicuousness be spread as it has the obvious cost by being more easily detected among cryptic preys. Another problem is that novel and uncommon prey may have a higher predation rate (antiapostatic selection) (e.g. Gamberale and Tullberg 1996, Lindstöm *et al.* 2001, Riipi *et al.* 2001). It was Bates (1862) who first described the relationship between an unpalatable model (a conspicuously colored butterfly) and an imitating unrelated palatable species (the

mimic). Later also Wallace (1867) and Darwin (1871) were puzzled by the phenomenon of aposematism. However, it was only in 1890 that Poulton coined the term aposematism the term aposematism defined it as “an appearance which warns off enemies because it denotes something unpleasant or dangerous; or which directs the attention of an enemy to some specially defended, or merely non-vital part; or which warns of other individuals of the same species”. Thus, aposematism is a composite concept consisting of two parts - a defense and a signal, with transformations within each (e.g. no defense to defense, see further paper IV). The current perception of aposematism is one of the signal, bright colors associated with a defense mechanism (e.g. a nasty sting, toxicity, aggressive behavior). However, the signal may also be warning odors, sounds, behaviors or electrical signals. The signal conveys information about the prey to the predator, thereby reducing energy costs for both the prey and the predator. Predators do not have to waste energy attacking unprofitable prey while the prey save energy by negating the need for escape behaviors. An effective signal must be easily recognizable, rapidly learned and remembered by potential predators (Harvey and Paxton 1981; see also Lindstöm 1999). Thus, warning signals benefit from being conspicuous.

Fish learn to avoid solitary unpalatable whirligig beetles (Benfield 1972) and presumably birds also learn to do the same. The dorsal sides of the whirligig beetles are black and more or less shiny and the ventral sides are often darkened brown or reddish. The black or dark coloration of the whirligig may be highly contrasting against the sky and/or the water surface, Black coloration *per se* is generally not considered a warning color, although Ohara *et al.* (1993) showed that the black color of the unpalatable sawfly larvae, *Athalia rosae* acted as a warning color when supplied together with palatable green butterfly larvae, *Pieris rapae* to naïve chicks, *Gallus gallus*. Thus it is possible that whirligig beetles are aposematic.

The warning signals of toxic insects are often “multimodal”, combining coloration with sounds and odors (or both) (Rowe and Guilford 1999). The whirligigs characteristic swimming motion and odor are potential additional signals. This typical motion pattern may increase the distance at which predators are able to identify the whirligigs, thereby reducing recognition errors (Guilford 1986, 1990). The whirligig species *G. minutus* used as prey in paper II and III seems to have a less conspicuous swimming behavior than for example *G. aeratus*, which also produces smaller waves

when swimming (Härilin pers. obs.) Its ventral sides are yellow and dorsal sides are dull, black. Furthermore, they do not aggregate as solitary species, but may join aggregation of other species e.g. *G. aeratus*. *Gyrinus minutus* may not be as conspicuous as other whirligigs and due to their lack of volatile secretion and a less functioning defense against fish (paper III) they will perhaps not benefit from warning signals and aggregating behaviors. Furthermore, *Gyrinus minutus* generally live in the emergent vegetated parts of lakes where fish predation probably is lower than in the open water. Maybe *G. minutus* habitat choice is an adaptation to avoid fish predation.

As mentioned previously, fish appeared indifferent to volatile odors as they attacked a new whirligig with the same vigor as after previous exposure to volatiles. Volatile taste appears to be the most effective single deterrent of potential fish predators (paper III). A study of novelty effects in a multimodal warning signal presented to domestic chicks, *Gallus gallus*, showed that novel odor was a more effective warning signal than color (Rowe and Guilford 1999). However, the whirligig beetle as a whole, including its swimming behavior, probably provides the most effective protection as Marples *et al.* (1994) showed for ladybirds.

Our experiments (paper III) on whirligigs revealed that group living species possessing volatile secretions were more easily caught by an invertebrate predator than were primarily solitary species containing no volatile substance. In contrast, my experiments (paper II) with fish predators showed that beetles with secretions containing both volatiles and norsesquiterpenes were more protected against fish predation than species whose secretion contained only norsesquiterpenes. This suggests that aggregating behavior may increase the effect of the strong-smelling volatile substance so that a combination of the aggregating behavior and the volatile substance may represent an enhanced aposematic signal (e.g. Sillén-Tullberg and Leimar 1988; Gagliardo and Guilford 1993; Lindström *et al.* 1999).

If not working with fish (see paper II) odor may work to potential bird predators. Aggregations on their own are also thought to be an aposematic strategy (Edmunds 1974, Gagliardo and Guilford 1993). Aggregated species need not be aposematic (e.g. Foster and Treherne 1980) and such species usually have very effective escape responses when encountered by a predator that allow early warning (Vulinec 1990). Nevertheless, whirligigs in an aggregation use early warning systems (Vulinec and Miller 1989). The whirligig species studied in this thesis seem to behave

similarly when approached by a predator also when not aggregated, but this needs further study. It seems that the lack of volatiles makes aggregating behavior unprofitable in whirligigs since without volatiles these species have no ability to use their secretion as an alarm substance (paper I) and they would probably have a higher risk of predation living in an aggregation than species producing volatile secretion.

Living in groups is risky for small prey if they are palatable, since a predator then may be able to eat all the individuals (Treisman 1975; Sillén-Tullberg 1988; Tullberg *et al.* 2000. Sillén-Tullberg and Leimar (1988) suggested that aggregation “dilutes” predation risks even in unpalatable prey. Still, the scentless whirligigs have some defense in their high molecular pygidial secretion, they are also defended by their fast zig zag swimming behavior, and yet they do not form aggregations. This defense and the dilution effect seem to be too weak to overcome the cost of establishing an aggregation in the whirligigs lacking volatiles.

Sometimes species lacking volatiles are found in aggregations of scenting species (e.g. *G. aeratus*) and even though species lacking volatiles cannot produce alarm substances they can react if exposed to them (paper I). Hence, unscented gyrids may take advantage of the scenting beetles alarm system and thereby escape approaching danger without own production of costly volatile secretion, i.e. they may have evolved a form of mimicry (see paper IV). It is also suggested that aggregation behavior evolved in already aposematic prey, in a phylogenetic analysis of butterfly larval and egg clustering the aggregating behavior has evolved after warning coloration and/or some kind of chemical and structural defense (Sillén-Tullberg 1988, 1993; Tullberg and Hunter 1996). These questions need phylogenetic answers and it is not yet known whether the production of volatile secretion is ancestral in *Gyrinus* and that some species (e.g. *G. minutus* and *G. opacus*) later have lost the ability to produce volatiles or whether the lack of volatiles is the ancestral condition for *Gyrinus* (question raised in paper I). Most studies of aposematism concern either the *function* of warning coloration (i.e. what makes the warning coloration effective as a signal and why is conspicuousness beneficial to unprofitable prey) or the *evolution* of aposematism (i.e. “how could warning signals arise, spread and become stabilised” (Gamberale-Stille 2000)). I am interested in both, but my main concern in this thesis lies in unifying aspects of aposematism into a historical and phylogenetic concept.

Towards a phylogenetic approach to aposematism (Paper IV)

It is generally believed that the evolution of unpalatability must precede the origin of warning signals to count as aposematic (Harvey and Paxton 1981; Guilford 1988; Sillén-Tullberg 1988; Guilford and Dawkins 1993; Alatalo and Mappes 1996; Lindström 1999; Tullberg *et al.* 2000). Selection pressures unrelated to defense, like sexual selection or thermo-regulation, may also select for conspicuousness in a species. Taxa achieving their conspicuousness for these reasons should not, according to Guilford and Dawkins (1993), be treated as aposematic since the conspicuousness has not been selected for a warning role. Unfortunately there is little empirical evidence for such evolutionary scenarios (paper IV Table 1, see also Summers and Clough (2001)), but it is at the heart of understanding aposematism. Functionalists sometimes argue that the direction of changes is irrelevant as long as the defense works. So it may be, but we hope to have shown that this is an impoverished view, and even if one were to allow all directions in an aposematic concept we strongly favor knowing *the* direction of events in order to be able to provide satisfactory explanations. That is, regardless how we restrict the concept of aposematism, knowing the directions of historical events facilitate all kinds of comparisons with a promise of uniting functional and evolutionary aspects into a historization of aposematism.

The historical view presented in this thesis (paper IV) aims at providing any student of aposematism with a method to interpret his or her observations within an explicit historical framework minimizing the risk of making less complete comparisons. Most of the attention directed towards understanding aposematism and its relatives (mimicry and crypsis) are based on learning experiments where predators are faced with "warning colored" prey. That is, a traditional and experimentally ecological approach -- an approach that basically is ahistoric since it lacks a phylogenetic component. Even though some experimental designs (e.g. Alatalo and Mappes, 1996) aims to test historical events, we agree with Tullberg *et al.* (2000) that no experimental studies can solve the evolution of aposematism. Rather, "deep phylogenetic insights would be needed" (Tullberg *et al.*, 2000). Although it has been recognised that "[p]hylogenies are fundamental to comparative biology ... [and] that there is no doing it without taking them into account" (Felsenstein, 1985) for quite some time, few studies take phylogeny into account (but see Sillén-Tullberg, 1988; Zrzavy, 1994; Tullberg and Hunter, 1996; Vogler and Kelley, 1998; Summers and

Clough, 2001) and there is clearly a need to bring the phylogenetic analyses even further. Tree-thinking (O'Hara, 1988, 1992) needs to become the hard core in evolutionary biology and not just an explanatory tool.

A primary step in such a process is to phylogenetically define the concept of aposematism and then proceed to infer such instances in the particular tree of interest. That is, before we can ask the question *how* aposematism has evolved we must know *where* it has evolved. To understand *why* a particular prey is successful in defending itself against predators we need to know the history of, and what caused, the defense and signal. A corollary of a pure functional aspect of aposematism is that the direction of the origin of unpalatability in relation to warning signal is irrelevant as long as the defense works against a predator. This is clearly an ahistoric view of biological problems and an approach that echo "state explanation" (O'Hara, 1988). Questions like "why are the coral snake brightly and conspicuously colored?" are often answered with state explanations like "because it is a warning signal indicating toxicity to predators" – an answer devoid of history and change. In order to transform the explanation into an event based explanation we need to provide an evolutionary scenario. An example of an event-based explanation could be "the coral snake's bright colors have evolved from cryptic colors as a result of selection for a warning role against predators".

To ask why certain species have a particular attribute is to suggest that that attribute is a derived character uniting them in a clade, and that the appearance of the character is the thing for which an explanation is sought. Likewise, the lack of the particular attribute in a group of species is to suggest that the attribute *per se* is primitive and has been lost – consequently it is the loss that needs an explanation (Fig. 1). Hence, it is important to keep in mind that a statement like "why an animal *is* warning colored" really should be read as "why it *has become* warning colored". This way of analyzing evolutionary questions is what O'Hara (1988) calls tree thinking.

Testing whether the hypothesised evolutionary scenario of aposematism is an adaptation we follow the general protocol on cladistic adaptation suggested by Coddington (1988). Briefly outlined such a test could look something like the following. When a transformation from lack of defense to a defense is followed by a switch from cryptic/non-conspicuous to conspicuous colours/signals is identified in a tree (Fig. 1) we need to take a look at the sister lineage where no such transformations

has occurred. At this stage we need to perform an experiment showing that our hypothesised aposematic lineage perform better against predators than their cryptic/non-conspicuous sisters do in their particular environment. Note that a phylogenetic hypothesis is needed also to find the most suitable controls, i.e. the closest sister taxa. If our scenario survives this test then we have a corroborated hypothesis of aposematism.

An adaptive hypothesis needs to be tested against a selective regime (Baum and Larson, 1991). The selective regime one identifies is contingent upon the particular hypothesis being tested. In the case of aposematism such a selective regime could be “distasteful to predators”. The first step is to optimize the selective regime on the tree showing that it is congruent with the transformations involved in our hypothesis of aposematism. For simplicity assume that taxa F-O (Fig. 1) share the selective regime “distasteful to predators”. After that, within such a selective regime we must show, experimentally, that a lineage like N or O have a higher survival rate than does M or L (Fig. 1). An objection sometimes raised in this context is that a lineage like M has adapted to its particular environment and thus makes a poor control for N since it does not control for divergence in ecology. We do not deny that changing ecology may be a problem, but it is not a problem unique for the phylogenetic approach. On the contrary, within a phylogenetic context it is possible to study the evolution of any aspect of biology using character reconstruction and outgroup comparison. Therefore, we argue that it is only within a phylogenetic context that we can control or address such things as changing environments. Using a phylogenetic approach we have the opportunity to optimize any character changes that we believe have a bearing on the hypothesis being considered.

Ideally, although difficult, within a phylogenetic approach adaptational experiments can be performed within an experimental design that mimics the ancestral states of interest. None of this means that a phylogenetic approach will necessarily provide us with the true answer to our questions. Phylogenetics, just like any other biological discipline, is a scientific endeavour dealing with hypotheses. However, the problems with reconstructing phylogenetic trees and ancestral character distributions (e.g. Losos 1999) are no excuse for not using phylogenetics in biology any more than the problems with experimental design and statistical analysis are excuses for not doing experiments. Scientifically, phylogenetic hypotheses are no worse or no better

than any experimentally derived hypothesis, but they do add the important dimension of evolutionary history.

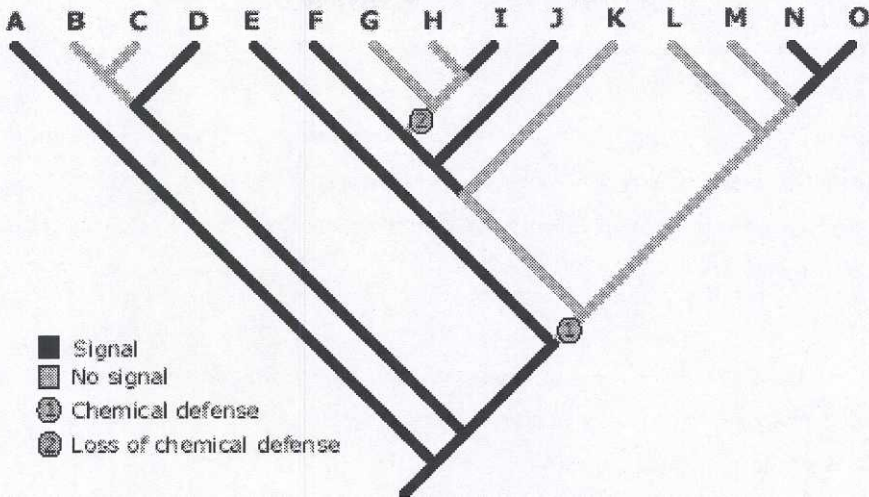


Figure 4. This tree provides a historical framework for identifying possible instances of aposematism, mimicry, and non-conspicuousness (crypsis). We have plotted the character states signal and no signal with the help of the MacClade 4.01 software (Maddison and Maddison, 2001) using ACCTRAN. The character states chemical defence and loss of chemical defence are also plotted. Based on these transformations we can identify two testable instances of aposematism (F/J and NO) with their possible controls (K, L, and M). However, as far as possible we stress the importance of choosing the closest sister taxon as control in order to minimise effects of taxon sampling, tree topology, and other historical effects. Hence, K is preferable as control for F/J and M is preferable for NO. For these tests of aposematism we also need to optimize a selective regime (Baum and Larson, 1991) like “distasteful to predators”. For simplicity, assume that taxa F-O share this selective regime. We also identify one testable case of Batesian mimicry (I) with its most suitable control (H) assuming that I resembles and live in sympatry with F or J as well as living in sympatry with G and H. More is involved in testing adaptive scenarios of mimicry (outside the scope of the

present paper) but an initial requirement is to show that the hypothetical mimic evolved after its likely model. Last, we identify three testable cases of non-conspicuousness (BC, GH, and KLM) with their most suitable controls (D for BC, E for KLM, F for GH). Note that non-conspicuousness in this case is both an instance of testable adaptation (KLM in relation to E) and a control for a test of a more derived hypothesis of aposematism (NO) – hence the need of tree-thinking all the way down the tree. Tree thinking also suggests that finding the ancestral state is dependent on the inclusivity of the tree. If we consider clade FO the ancestral state is no signal (non-conspicuousness/crypsis) but if we consider the more inclusive clade AO the ancestral state is signal (but no defence).

To be able to test the hypothesis of aposematism in whirligig beetles we need information on the distribution of aggregations and volatiles (in progress) and a phylogenetic hypothesis (paper V).

***Gyrinus* Phylogenetics (Paper V)**

The knowledge of the *Gyrinus* phylogeny is sparse. Oygur and Wolfe (1991) have presented the only phylogenetic analysis of *Gyrinus* to date. Their study was based on 22 morphological characters (both multistate and ordinary binary) and 40 Nearctic species of *Gyrinus* with *Spanglerogyrus albiventris* as outgroup and resulted in a rather unresolved hypothesis. In the present study we take a slightly different approach based on the morphological variation presented by Oygur and Wolfe (1991, and references therein) and our own studies. We let an extensive outgroup analysis decide the polarity and transformation of the characters (Nixon and Carpenter, 1993). Oygur and Wolfe (1991) used a two-step analysis where they analyzed the relationships between the Gyrinid genera in one analysis and among the species of *Gyrinus* in another analysis. Hence, since they used only a single outgroup for the *Gyrinus* analysis they did not evaluate the monophyly of *Gyrinus* in any severe manner. In paper V we assess the monophyly of *Gyrinus* by including members from *Aulonogyrus* (2 species), *Orectochilus* (2 species), *Dineutus* (3 species) and *Spanglerogyrus* (1 species) in one simultaneous analysis. We have also increased the number of *Gyrinus* species to 47. Following the coding scheme suggested by Pleijel (1995), we have recoded the morphological variation presented by Oygur and Wolfe

(1991, their Tables 8, 9 and 12) into 250 absence/presence characters. Since absence/presence characters potentially allow for more direct homology tests we believe this coding better suited to evaluate the phylogeny of *Gyrinus*.

Oygur and Wolfe (1991) (from now on referred to as O&W) presented a final consensus tree (their figure 162) consisting of three major polytomies of which only one by necessity was monophyletic (their clade 3). The other two (referred to as clade 1 and 2 by O&W) could equally well be non-monophyletic. In addition to their final consensus tree, O&W also present a slightly more resolved consensus tree (their figure 161). This latter tree is based on a manipulation with the original data set involving deletion of all identical taxa as well as taxa differing by one or two characters. As a consequence that tree is based on only 29 species instead of 40. There are several problems with attaching species to a tree after an analysis (Härilin 1999), not the least being that these species have not participated in the congruence analysis and therefore cannot influence tree topology.

The monophyletic polytomy in O&W's final consensus tree includes the following species: *pleuralis*, *pectoralis*, *parcus*, *dubius*, *hoppingi*, *opacus*, *rugosus*, *gibber*, *pernitidus*, *piceolus*, *picipes*, *analis*, *dichorus*, *marinus*, *wallisi*, *aeratus*, *impressicollis*, *affinis*, *pugionis*, *borealis*, and *sayi*. These species are all, except *dichorus* (clade A) and *rugosus* (clade C), present in our clade B (Fig. 5). Clade B also includes *maculiventris* which is in full agreement with the hypothesis in O&W since that species belongs to a polytomy just outside their monophyletic polytomy and hence could, had their relationships been better resolved, be a part of that clade. In addition, our clade B includes four species (*natator*, *caspius*, *paykulli*, and *distinctus*) not present in O&W's hypothesis. So we are in almost perfect agreement with O&W regarding the content of clade B and, as an extra bonus, clade B is fully resolved in our hypothesis (Fig. 5).

The basal polytomy in O&W contains the species *latilimbus*, *ventralis*, *fraternus*, *bifarius*, *obtusus*, *rockinghamensis*, *minutus*, *confinis*, *pachysomus*, *elevatus*, *plicifer*, *marginellus*, *woodruffi*, and *aeneolus*. O&W is inconclusive whether this polytomy is monophyletic or not. According to our hypothesis, this polytomy is polyphyletic since those species are split between clade A and C (Fig. 5, paper V). We suggest that *aeneolus*, *elevatus*, *latilimbus*, *dichorus*, *minutus*, *rockinghamensis*, *gehringi*, *ventralis*, *bifarius*, *confinis*, *obtusus* and *fraternus* belong in clade A and is

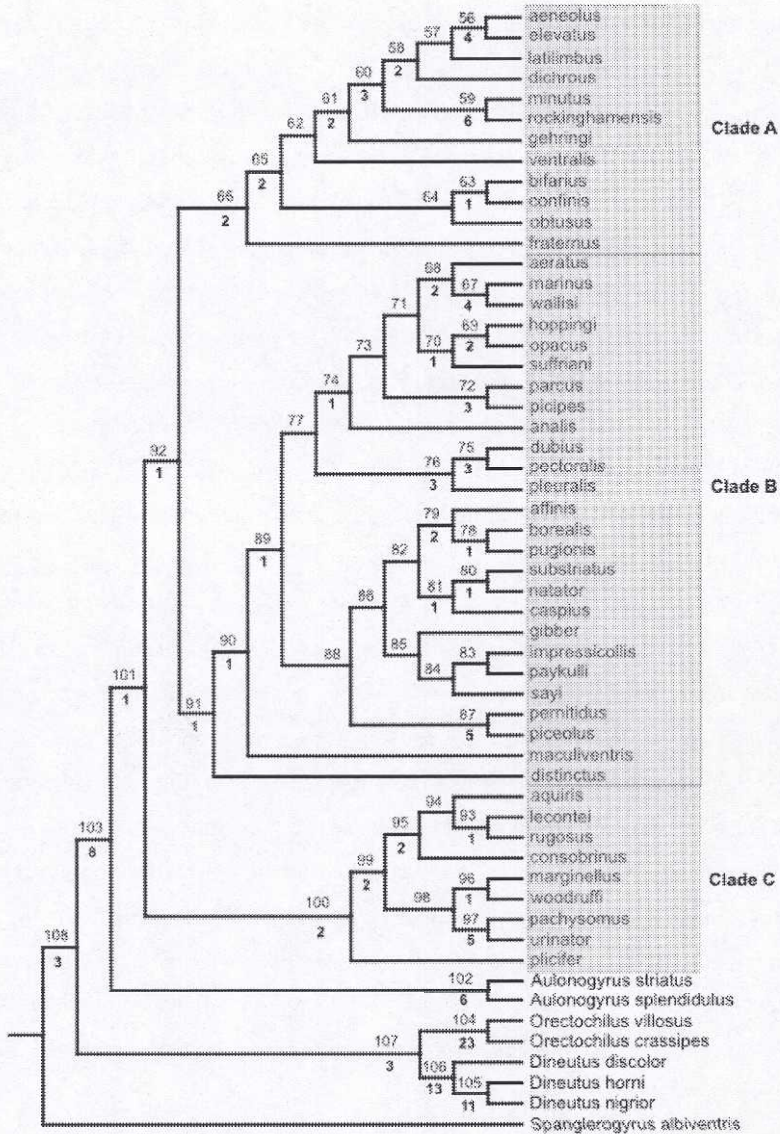


Figure 5. Phylogenetic hypothesis of Gyrinus (from paper V). Bremer support values are given below each branch in bold face. Numbers above branches are the node numbers.

the sister group to clade B. In clade C, which is the sister group to A and B collectively, we find *aquiris*, *lecontei*, *rugosus*, *consobrinus*, *marginellus*, *woodruffi*, *pachysomus*, *urinator*, and *plicifer*.

Many of the minor clades present in O&W like *minutus* - *rockinghamensis*, *aeratus* - *marinus* - *wallisi*, *hoppingi* - *opacus*, *borealis* - *pugionis*, and *pernitidus* - *piceolus* are also present in our study. But there are also major differences like the species pair *woodruffi* - *aeneolus* present in O&W that is split between clade A and C in our hypothesis.

Compared to the hypothesis in O&W our study has basically supported and resolved certain parts of O&W like clade B in our Figs. 1 and 2 (paper V). In addition we have resolved O&W's other two polytomies into two non-overlapping clades (A and C, in Figs. 1 and 2, paper V). Note that this is not in conflict with O&W it just represents one solution of all possible solutions included in O&W's hypothesis. We believe that with our study we have taken yet another step towards understanding *Gyrinus* phylogeny. The hypothesis presented here will provide a framework for testing evolutionary scenarios as well as the basis for future combinations with molecular data.

Concluding remarks

The major conclusions from this thesis can be divided into two parts, one empirical concerning predation experiments and phylogeny, and one theoretical concerning the concept of aposematism. From the experiments one can conclude that volatile secretions play multiple roles in whirligig anti-predator behavior. For instance, they function as alarm substances among individuals in aggregations. The volatiles (rather than the norsesquiterpenes) also seem to be the more effective component (perhaps in combination with norsesquiterpenes) in the whirligigs predator defense against fish. Furthermore, preliminary field observations suggest that only whirligigs having volatiles show a strong tendency to aggregate. However, a phylogenetic approach with a large number of *Gyrinus* species is needed to address the evolutionary relationship between aggregating behavior and the production of volatiles. My experiments also show that the invertebrate *Notonecta glauca* is a potential predator on whirligig beetles and that species of whirligigs differ in vulnerability to backswimmer predation. The final empirical part of my thesis concerns the phylogeny

of *Gyrinus*. Using a strict absence/presence coding of morphological characters, which allows for more direct homology assessments, I present a fully resolved hypothesis of 47 *Gyrinus* species.

In the theoretical part I developed a phylogenetic approach to the concept of aposematism stressing the historical nature of evolutionary concepts. Concepts, like aposematism, involving change are beneficially treated as historical concepts of events.

The next step in understanding whirligig evolution will be to unite the phylogenetic hypothesis with the theoretical model of aposematism and empirical data on distribution of aggregations and volatile secretes. This, together with more experiments, provides the basis for evaluating the hypothesis of aposematism in whirligig beetles. With the present thesis I have taken the first steps in that direction.

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