Taxonomy, phylogeny, and secondary sexual character evolution of diving beetles, focusing on the genus Acilius

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Johannes Bergsten

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Fakultetsopponent: Docent Jyrki Muona, Division of Entomology, Zoological Museum, University of Helsinki, Finland

Department of Ecology and Environmental Science
Umeå University
SE-90187 Umeå, Sweden
Sexual conflict can lead to antagonistic coevolution between the sexes, but empirical examples are few. In this thesis secondary sexual characters in diving beetles are interpreted in the light of sexual conflict theory. Whether the male tarsal suction cups and female dorsal modifications are involved in a coevolutionary arms race is tested in two ways. First eight populations of a species with dimorphic females that varied in frequency of the morphs were investigated and male tarsal characteristics quantified. The frequency of female morphs is shown to be significantly correlated to the average number and size of male tarsal suction cups in the population, a prediction of the arms race hypothesis. Second, the hypothesis is tested in a phylogenetic perspective by optimizing the secondary sexual characters on a phylogeny. A full taxonomic revision of the genus *Acilius* is presented, including new synonyms, lectotype designations, geographic distributions based on more than five thousand examined museum specimens and the description of a new species from northeastern USA. Specimens of all species (except one possibly extinct that failed to be found in Yunnan, China 2000), were field collected between 2000 and 2003 in Sardinia, Sweden, Russia, Honshu and Hokkaido in Japan, New York, Maryland, California and Alberta. Three genes (CO1, H3 and Wingless) were sequenced from the fresh material as well as scoring a morphological character matrix all of which was used to derive a robust and complete hypothesis of the phylogenetic relationship in the group. The phylogeny was derived using Bayesian phylogenetics with Markov Chain Monte Carlo techniques and received a posterior probability of 0.85. Changes in male and female characters turned out to be perfectly correlated across the phylogeny, providing one of the best empirical examples to date of an antagonistic arms race between the sexes in a group of organisms. Finally, a review of a pitfall to phylogenetic analysis known under the name long-branch attraction (LBA), is provided. The problem is well known theoretically but has been questioned to occur in real data, and LBA has been in the core center of the hard debate between parsimony and likelihood advocates since different inference methods vary in sensitivity to the phenomenon. Most important conclusions from the review are; LBA is very common in real data, and is most often introduced with the inclusion of outgroups that almost always provide long branches, pulling down long terminal ingroup branches towards the root. Therefore it is recommended to always run analyses with and without outgroups. Taxon sampling is very important to avoid the pitfall as well as including different kind of data, especially morphological data, i.e. many LBA-affected conclusions have recently been reached by analyses of few taxa with complete genomes. Long-branch extraction (incl. outgroup exclusion), methodological disconcordance (parsimony vs modelbased), separate partition analyses (morphology vs molecules, codon positions, genes, etc), parametric simulation (incl. random outgroups), and split graphs are available relevant methods for the detection of LBA that should be used in combinations, because none alone is enough to stipulate LBA.

**Key words:** antagonistic coevolution, arms race, sexual conflict, diving beetle, Dytiscidae, Coleoptera, taxon sampling, long-branch attraction, parsimony, Bayesian analysis, taxonomic revision

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Johannes Bergsten

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Department of Ecology and Environmental Science
Umeå University
SE-90187 Umeå, Sweden
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"De fria fåglarna plöja sin väg genom rymden. Många av dem nå kanske ej sitt fjärran mål. Stor sak i det. De dö fria."
(Torgny Segerstedt, 1940)
List of papers

The thesis is based on the following papers which will be referred to by their roman numerals;


IV. Bergsten, J. & Miller, K.B. Female diving beetles in antagonistic coevolutionary arms race with male suckers (*Manuscript*)

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All new names and nomenclatural acts in this thesis are disclaimed for the purpose of zoological nomenclature (Articles 8.2, 8.3 ICZN).
Background
Diving beetles (family Dytiscidae) show a range of peculiar secondary sexual characters in both males and females. Males have their three basal segments of the pro- and often also the mesotarsi equipped with various combinations of small and large suction cups (Fig. 1). In spite of early speculations that these cups can be used for climbing, swimming and catching prey, it has long been recognised that their main purpose is connected with reproduction (see Blunck, 1912a).

Figure 1 Male protarsal palette: A: *Dytiscus dimidiatus*, B: *Hydaticus decorus*, C: *Graphoderus austriacus*, D: *Acilius sulcatus*, E: *Graphoderus zonatus verrucifer*, and F: *Thermonectus marmoratus*. 
The male places his fore feet on the female pronotum or anterior third of elytra, initially during mating interactions (Blunck, 1912b; Régimbart, 1877; Aiken, 1992). Already Blunck (1912a) recognised that if deprived of his suction cups a male would not be able to accomplish matings. Since accomplishing matings is the only way to have a fitness larger than 0, i.e. to spread his genes on to the next generation, it seems clear that this is the main force of selection. Had the loss been as detrimental (fitness necessarily 0) for catching prey, swimming or climbing, then the lack of tarsal suction cups in females is unexplainable. Darwin’s ideas on sexual selection thus seems most appropriate as explanatory theory. The brilliant naturalist as he was, diving beetles and their spectacular characters had not escaped his evolutionist’s eye, but was in fact used as an example when he presented his theory of sexual selection. In his tenth chapter entitled ‘Secondary sexual characters of insects’ he gives an explanation to diving beetle characters (Darwin, 1871:286):

“The tarsi of the front-legs are dilated in many beetles, or are furnished with broad cushions of hairs; and in many genera of water-beetles they are armed with a round flat sucker, so that the male may adhere to the slippery body of the female. It is a much more unusual circumstance that the female of some water-beetles (Dytiscus) have their elytra deeply grooved, and in Acilius sulcatus thickly set with hairs, as an aid to the male. The females of some other water-beetles (Hydroporus) have their elytra punctured for the same purpose.”

Not only have males suction cups to grab the slippery body of females, but the females have variously modified elytra “as an aid to the male”, according to Darwin. This earlier view of harmony and cooperation in the production of offspring was not challenged until much later (e.g. Parker, 1979) and only in the last 15 years has a new field, devoted to explore the various conflicts existing between the sexes, formally exploded (review in Chapman et al. 2003; special issue S5 vol. 165 of American Naturalist, May-2005).
The female characters in diving beetles thus constitute variously modified pronotum and elytra. Observed modifications include furrows, hairs, punctures, impressed lines in reticulate networks, granulations, wrinkles, hash marks etc. on areas where the male is usually completely smooth and shining (Fig. 2).
Cybister 102 species Global

Cybister lateralimarginalis

Thermonectus 18 species N. & S. America

Thermonectus basillaris

Dytiscus 27 species Holarctic

Dytiscus lapponicus
Figure 2 Contrasting male and female sculpture on pronotum and elytra in species representing seven different genera of Dytiscinae.
Apart from Darwin’s ideas of their purpose and function, a few others have been proposed. Sharp (1882) noted geographical variation in the distribution and frequency of modified female morphs and consequently gave climatic factors as a possible explanation, without detailing the explicit process or function. Roughley and Pengelly (1981) likewise focused on differences in distribution between morphs and suggested they resulted from different glacial refugia and subsequent recolonization. Wesenberg-Lund (1912) gave a completely different hypothesis. He noted how a furrowed compared to a smooth back breaks the water surface differently when the beetle comes to the surface for replenishing the air-supply under the elytra. Thus the ridges had, according to Wesenberg-Lund, a function to strengthen the force with which the water surface tension carries a beetle, and explained the occurrence solely in females of the furrows, as this extra carrying capacity is most important for heavy, egg-filled, females. Guignot (1931-33) rejected all previous explanations except that of Camerano (1880) and thus favoured an adaptationist chauvinistic hypothesis where females were just slower than males to adapt to the aquatic environment. He considered the elytral furrows to be ancestral and slowly disappearing from aquatic diving-beetles.

In light of the recent fundamental twist in the understanding of male-female interactions where terms like polygamy, mating costs, coercive matings, precopulatory struggles, antagonistic arms race; in short - sexual conflict, are here to stay, it was time to reinterpret the secondary sexual characters in diving beetles.

Introduction

From sexual conflict to arms race
Conflict between the sexes in mating systems seems to be much more widespread than previously believed (Chapman et al., 2003). Sexual selection in terms of female choice and male-male competition has been the dogma for a long time, probably caused by an overestimation of harmony and lifelong monogamy in nature. Polygamy sets the stage for sexual conflict (Arnqvist & Nilsson, 2000), the importance of which as an evolutionary driving force is only in its infancy of being comprehended. However, it has already been identified as a very important mechanism of speciation, being an engine of rapid evolutionary change in reproductive physiology and behaviour (Arnqvist et al., 2000; Gavrilets, 2000; Gavrilets and Waxman, 2002; Knowles and Markow, 2001; Martin and Hosken, 2003; Parker and Partridge, 1998; Rice, 1996, 1998; Rice and Holland, 1997). Anisogamy, the difference in size between the male and female gametes and thereby the differential investment in each offspring by the sexes, is the bottom line of the conflict (Parker, 1979; Alexander, 1997). In general for males to increase their fitness there is a strong selection for multiple matings, the more females they mate the more offspring they sire. Females on the other hand often have a much lower optimal mating frequency, one or a few matings are usually enough to fertilize all their eggs (Arnqvist, 1997), and if additional mating does not coincide with nuptial gifts, access to resources etc (Hammerstein & Parker, 1987), they are more costly than beneficial for the female. Matings can be costly due to increased risk of predation (Rowe, 1994; Arnqvist, 1989a), direct genital injury (Blanckenhorn et al., 2002; Crudgington and Siva-Jothy, 2000; Stutt and Siva-Jothy, 2001), energy expenditure (Watson et al., 1998), decreased foraging ability (Rowe, 1992),
increased risk of diseases (Hurst et al., 1995) and hormones and toxic substances in the male seminal fluids affecting the female in the best interest of the male as opposed to her own best lifelong fitness (Fowler & Partridge, 1989; Chapman et al., 1995). Males share some of the same costs but have a much larger potential benefit from engaging in copulation. Thus, in any given situation of male-female interaction, the male will be under selection to mate, while the female will be under selection not to mate (Parker, 1979). The outcome of the conflict is theoretically expected to be an antagonistic coevolutionary arms race between the sexes (Parker, 1979, 1984; Alexander et al., 1997; Cluttonbrock and Parker, 1995). The arms race can be carried out at the molecular level (Chapman et al., 1995; Fowler & Partridge, 1989; Rice, 1996), on a signaling-perception/reception system (Holland & Rice, 1998), in behaviour (Arnqvist, 1989b; Crean & Gilburn, 1998), in morphological adaptations and counteradaptations (Arnqvist & Rowe, 2002; Paper I) or in any combination of the above.

An evolutionary arms race needs a genetical basis and here we differentiate between intralocus and interlocus conflict (Rice, 1998; Chapman et al., 2003). Intralocus conflict is defined as when the gene product of a certain locus, expressed in both sexes, has a different optimum between the sexes. Here what is selected for in one sex may be counterselected for in the other sex. Interlocus conflict is when characters expressed at different loci and only in either sex affect the outcome of a male-female interaction, the result of which imposes a negative fitness on the looser. In this case each sex can reach its fitness optimum of the expression, even though the outcome of the interaction remains open. Sex chromosomes have been a focus of interest in this regard (Rice, 1984, 1998; Gibson et al., 2001) but also autosomal chromosomes can house genes with sex specific expression by other mechanisms (Rhen, 2000). The underlying genetic bases of most traits are still unknown, but empirical examples of intersexual arms race start to accumulate. In Drosophila elegant experimental setups have revealed the antagonistic evolution between the sexes, showing how one sex can evolve to increase its fitness even though, counterintuitively, as a consequence, the lifetime and reproductive fitness of its partner is reduced (Civetta & Clark, 2000; Rice, 1996). The conflict can often take the shape of premating struggles and forced copulations (Cluttonbrock & Parker, 1995; Thornhill, 1980). Waterstriders (Arnqvist, 1989b, 1992), scorpionflies (Thornhill, 1980; Thornhill & Sauer, 1991) and crickets (Sakaluk, et al., 1995) are some examples where male-specific morphological characters have been showed in experiments to function as a way of coercing unwilling females to mate, contrary to the idea that it is a way of female choice. Females on the other hand have developed behaviors in several groups to get rid of harassing males; shaking (Allen & Simmons, 1996; Blanckenhorn et al., 2002), kicking (Crean & Gilburn, 1998; Jormalainen & Merilaita, 1995), somersaulting (Rowe et al., 1994; Arnqvist, 1997), erratic swimming behaviour (Aiken, 1992; Bisazza et al., 2000) etc. When it comes to morphological female counteradaptations examples are very scarce but a pair of abdominal spines in a waterstrider has been shown to function as a harassing male-disloder (Arnqvist & Rowe 1995) and Paper I suggested that various examples of female dorsal modifications in diving beetles interfere with male grasping devices. However coevolutionary studies of traits or behavior should preferably, not only include but, be carried out in a phylogenetic perspective.
To date there is a disproportionate lack of studies addressing sexual antagonistic coevolution in a phylogenetic perspective. Exceptions include Andersen (1997) who searched for coevolutionary patterns between male clasping legs and genitalia and female anticlasp devices over a phylogeny of waterstriders, but did not find any such conclusive pattern. Clearly a more detailed homology-based investigation was needed as opposed to lumping a number of different characters into “female anticlasp devices; present/absent”. Arnyqvist & Rowe (2002) used instead quantitative morphometric data and independent phylogenetic contrasts (Felsenstein, 1985) across 15 species of one genus of waterstriders and showed that male and female abdominal modifications coupled to the premating struggle were significantly correlated. Poor taxon sampling (15 out of 42 known species in the genus) while assuming equal branch-lengths are however a potential source of misguidance in their study. In a phylogenetic study Miller (2003) tested the sequence of evolution of major male and female traits coupled to the arms race theory in diving beetles and found it to be as predicted; the evolution of various female dorsal modifications in different lineages postdated the evolution of effective male grasping devices. However this study was not detailed enough to show closely coupled coevolutionary events of male versus female characters but rather focused on the sequence of major novelties. Other interspecific comparative studies with a positive outcome of predictions from sexual antagonistic coevolution have focused on male sperm size versus female reproductive tracts, (Briskie et al., 1997; Morrow & Gage, 2000; Presgraves et al., 1999) but the role of sexual conflict is here unclear. Antagonistic evolution, having been shown both theoretically (Gavrilets, 2000) and empirically (Rice, 1996) to create rapid evolutionary change, predicts that those changes in male and female characters can be traced down to coincidental character transformations on internal nodes on a phylogeny. Clearly there is a need for phylogenetic approaches to the study of sexual conflict so far dominated by experimental approaches.

Diving beetles as study organisms

Diving beetles (family Dytiscidae) are an important group of predatory aquatic insects, most diverse in stagnant waters (Kehl & Dettner, 2003; Lundkvist et al. 2003). Diving beetles include 3892 recent species worldwide (Nilsson, 2001, 2003, 2004) and range from a mere millimetre in size up to almost 5 centimeters (Fig. 3).

Figure 3. Dwarfs and giants among diving beetles (Dytiscidae). Left *Hydroporus scalesianus*, right *Dytiscus latissimus*. Scale bar=30mm in total (1mm each line)
Despite this huge range in size, the form of a streamlined body is very similar throughout the family as an adaptation to the aquatic medium and good swimming ability. The diving beetles belong to the adephagous suborder of Coleoptera where several other aquatic families are found. Excitingly, two new families of aquatic adephagous beetles have recently been described from South Africa (Ribera et al., 2002a), China (Balke et al., 2003) and Venezuela (Spangler & Steiner, 2005). The question of whether these aquatic families “Hydradephaga” are monophyletic or not and thereby if the aquatic medium has been invaded once or several times independently in this suborder has been thoroughly debated (reviewed in Shull et al., 2001) but recent studies support a single origin (Ribera et al., 2002a,b; Shull et al., 2001).

The higher relationships within Dytiscidae have recently been the subject of several studies, both molecular (Ribera et al., 2002b) morphological (Miller, 2001) and combined (Miller, 2003), resulting, among other things, in the description of a new subfamily (Miller, 2001). The genera *Acilius*, *Graphoderus* and *Dytiscus*, the main focus of this thesis, belong, together with most of the large-size diving beetles, to the nominal subfamily Dytiscinae. The intertribal and intergeneric relationship within the subfamily have been investigated both morphologically (Miller, 2000) as well as combined with several genes (Miller & Bergsten, in prep., meeting abstract Bergsten & Miller, 2004).

The mating system: attack without courtship
The male suction cups are used to grip and secure the holding of a female at the initial contact before copulation (Régimbart, 1877; Blunck, 1912a; Aiken, 1992; Adis, 1974; Fig. 4). No courtship has been observed to proceed mating attempts and females are commonly resisting upon contact of a male, swimming fast and erratically away down into the vegetation or bottom substrate in an attempt to dislodge the male (Régimbart, 1877; Blunck, 1912; Aiken, 1992; Aiken & Khan 1992; pers. obs.). This precopulatory struggle is indicative (but not decisive) of sexual conflict and thus we have potentially the fuel present in this system for an arms race. If the male can outride the initial female attempt to get rid of the male, mating almost always occurs (Aiken, 1992). This means that, apart from postcopulatory mechanisms, it is in the few critical moments from contact to a secure grip that selection can act on arms race related morphology in males and females and it is in this perspective that Paper I envisioned the male tarsal suckers and female dorsal modifications play the main roles.

The male strikes his tarsal suckers on the female pronotum or upper part of the elytra (Régimbart, 1877; Blunck, 1912b; Wesenberg-Lund, 1912; Aiken, 1992) and the suckers have been shown to be able to lift many times the weight of a female (Plateau, 1872; Simmermacher, 1884; Törne, 1910; Aiken & Khan 1992). Notably it is in exactly the region subject for male tarsal adherence that we find a variety of structural modifications in females of different genera (Fig. 2; Paper I; Miller, 2003), all with the effect of making the dorsal surface more rough and uneven. Considering that the male suckers work mechanically (Plateau, 1872; Simmermacher, 1884; Euscher, 1910; Frankenberg, 1935; Aiken & Khan 1992), smooth flat surfaces are optimal for grip, while water leakage impedes the function when the cup cannot get full contact around the circumference and create a negative pressure underneath because of the uneven surface (Plateau, 1872; Simmermacher, 1884; Guignot, 1931-33; Frankenberg, 1935).
Figure 4 A mating couple of *Acilius sulcatus*. Male on top, female below. The time in copula lasts approx. 2.5 hours (unpubl. data), during which the female is immobilised and unable to replenish her air supply.
Aim of the thesis

If sexual conflict drives the evolution of antagonistic arms race between the sexes in diving beetles, then the prediction is that traits involved in the arms race should correlate. This can be tested in two different ways.

♦ On a micro evolutionary scale, the unique female dimorphism in some diving beetle species offers an opportunity to test the prediction. This can be done by comparing multiple populations, differing in frequency of the two female morphs and quantifying variation in male tarsal characteristics. (Paper I)

♦ On a macro evolutionary scale to test any evolutionary scenario, spanning geological timescales, a phylogenetic perspective is necessary. A suitable genus, *Acilius*, of diving beetles was chosen, suitable in the respect of showing variation in expression of secondary sexual characters across species. The aim was to derive a robust phylogeny of the extant species, optimize characters presumed to be involved and reject or confirm that males and females have coevolved in characters working antagonistically. (Paper IV)

♦ No modern revision existed for the genus *Acilius*, and species limitations and number of extant species was, especially in North America, poorly understood. Taxonomic revisions are very important in itself as the basic tool to describe the biological diversity on earth, and as the necessary basis for ecological, evolutionary or phylogenetic research on the group. Thus, to get to the point of testing the arms race hypothesis on the phylogeny, there was first a need of revising the genus. A full modern taxonomic revision of the genus *Acilius* was first undertaken. (Paper II)

♦ Second, after a revision has been undertaken and data have been collected for the phylogenetic analysis, there is always a concern to correctly apply phylogenetic methods, given the plethora of options available. In particular there are many methodological pitfalls that can cause erroneous conclusions. Since the final test of the arms race hypothesis will depend on the inferred phylogenetic hypothesis this is well worth seriously considering. Initial analyses suggested that the phenomenon, or pitfall, known as long-branch attraction (LBA) was confounding the analyses. Although theoretically, LBA is well understood, the existence of the phenomenon in real data analyses has been questioned and detection and avoidance methods suggested to date gave a poorly coherent message. Consequently a review of the phenomenon LBA was needed focusing on methods to detect and avoid LBA and bringing to light how common the phenomenon really is in real case data (as opposed to hypothetical as still suggested in modern student text-books; Schuh, 2000). (Paper III)
Material and methods

With a strict holarctic distribution of the genus *Acilius*, fieldwork was restricted to the Northern Hemisphere (Fig. 1). Collecting methods used were hand net and baited bottletraps. Beetles were killed and preserved in 95% ethanol. Material for paper I was collected in the four Swedish provinces Skåne, Medelpad, Västerbotten and Norrbotten.

![Map showing geographical localities](image)

Figure 5. Field work was carried out in the areas marked by a ring. Attempts to collect fresh material and habitat data of all extant species of *Acilius* were successful except for the Chinese species *A. sinensis*. This species might possibly be extinct. *Acilius duvergeri* was searched for both in Portugal and on Sardinia but found only in the latter place. The geographical localities represent from left to right: Sierra Nevada mountains (northern California, USA), central and southwestern foothill mountains of Alberta (Canada), upstate New York (USA), Maryland (USA), Odemira (Portugal), Sardinia (Italy), Västerbotten (Sweden), Volgograd Oblast (south european territory of Russia), Yunnan (China), Fukui prefecture (Honshu, Japan) and Hokkaido (Japan).

For the taxonomic revision material from the following museums were either examined at the museum or via loans.

**Visited Museums:**

- AMNH American Museum of Natural History New York, USA.
- CNC Canadian National Collection of insects, arachnids and nematodes, Ottawa, Canada.
- CUIC Cornell University Insect Collections, Ithaca, NY, USA.
- KIZ Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China.
- UCDC Bohart Museum of Entomology, University of California, Davis, California, USA.
- USNM National Museum of Natural History, Smithsonian Institution, Washington DC, USA.
- ZIN RAN Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia.
Material examined via loans:
CAS California Academy of Sciences, California, USA.
FSCA Florida State Collection of Arthropods, Gainesville, Florida, USA.
IRCW Insect Research Collection, University of Wisconsin-Madison, Madison, WI, USA.
IRScNB Institut Royal des Sciences Naturelles de Belgique, Bruxelles, Belgium.
MCZ Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts USA.
TAMU Texas A & M University, Texas, USA.
ZMUM Zoological Museum of M.V. Lomonosov Moscow State University, Moscow, Russia.
ZSM Zoologische Staatssammlung, München, Germany.

Taxonomic revision procedures were as standard practice (Winston, 1999; ICZN, 1999). Lectotypes were designated where no previous holotype, neotype or lectotype existed for a valid name, and unambiguous type material was retrieved at museum collections, with the expressed purpose of stabilising nomenclature to the most common usage of names. This agrees with the amended article 74.7.3 (ICZN, 1999) and recommendation that lectotypes should not be designated merely for curatorial purposes (ICZN, 2003 declaration 44).

DNA amplification and sequencing were done by my coauthor Kelly B. Miller at the DNA Sequencing Center, Brigham Young University, Provo, Utah. This followed standard protocols (see paper IV).

Bayesian and parsimony analyses were done on a Dell Inspiron 9100 P4 3,4GHz processor and 2GB RAM memory laptop. Phylogenetic analysis software used were MrBayes ver. 3.04b (Ronquist & Huelsenbeck, 2003) for Bayesian analyses and Winclada (Nixon, 1999-2002), Nona (Goloboff, 1999) and TNT (Goloboff, Farris & Nixon, 2000) for parsimony analyses.

Suction cup measurements were done by taking digital photographs (Kodak Megaplus camera Model 1.6i) through an Olympus microscope at 64x and imported to the image analysis software Optimas 6.5 (Media Cybernetics, 1987-1999). Illustrations were produced in a similar way or directly with a camera lucida connected to a Leica MZ95 dissecting microscope. Larger body measurements were taken by an ocular eyepiece with a scale bar.

The literature search for the review paper used the Web of Science database at http://isi3.newisiknowledge.com/portal.cgi/wos. The Treebase database at http://www.treebase.org/treebase/index.html were used for the download of data matrices.

Statistical analyses used included PLS (Partial Least Square, also known as Projection to Latent Structure) in paper I and the application of Bayesian statistics to the inference of phylogeny using Markov Chain Monte Carlo methods in papers III and IV. PLS is a technique that combines aspects from PCA (principal component analysis) with multiple linear regression. A set of variables are assigned to an X-matrix (independent) and another set to the Y-matrix (dependent). The PLS-analysis
then fits a hyperplane that both approximates the X and Y spaces and maximizes the correlation between them. Orthogonal principal components are extracted with variables receiving loadings and observations receiving scores, as in PCA, but in addition the degree to which X can predict Y is calculated with a significance level of the correlation.

The origin of Bayesian applications to phylogenetic inference was nicely summarised by Huelsenbeck et al. (2002), who also provide the basic explanation of Bayes theorem applied to phylogenetic trees and using Markov Chain Monte Carlo techniques to sample the joint posterior probability distribution. Bayes theorem

\[
\Pr[H|e] = \frac{\Pr[e|H] \times \Pr[H]}{\Pr[e]}
\]

where H is the hypothesis and e is the evidence, can applied to phylogenetic trees be written as

\[
\Pr[T_i|X] = \frac{\Pr[X|T_i] \times \Pr[T_i]}{\sum_{j=1}^{B(s)} \Pr[X|T_j] \times \Pr[T_j]},
\]

where Ti is the i:th phylogeny, X is the data matrix and B(s) is the total number of possible phylogenies for s taxa. We want to know \(\Pr[T_i|X]\) i.e. the posterior probability of tree \(i\) conditional of the data. The nominator is the likelihood of the data given tree \(i\), times the prior probability of tree \(i\). The denominator is a nasty summary of the likelihood times the prior of every possible tree. Remember that the possible number of trees grows rapidly with the increase of number of taxa,

\[
B(s) = \frac{(2s-3)!}{2^{s-2}(s-2)!},
\]

so that already 15 taxa can be related in almost 8000 billion different ways. In addition the likelihood for tree \(i\), \(\Pr[X|T_i]\), needs to be integrated over all possible values of branch lengths \(v\), and substitution model parameters in the vector \(\theta\);

\[
\Pr[X|T_i] = \int_{v,\theta} f(X|T_i, v, \theta) f(v, \theta) dv d\theta
\]

\(f(v, \theta)\) is here the function of the priors for branch lengths and substitution model parameters. Thus the necessary calculation to get the posterior probability of tree \(i\),

\[
\Pr[T_i|X] = \frac{\left[ \int_{v,\theta} f(X|T_i, v, \theta) f(v, \theta) dv d\theta \right] \times \Pr[T_i]}{\sum_{j=1}^{B(s)} \left[ \int_{v,\theta} f(X|T_j, v, \theta) f(v, \theta) dv d\theta \right] \times \Pr[T_j]},
\]
is impossible analytically, why the numerical technique known as Markov Chain Monte Carlo (improved by Metropolis Coupling) is typically used to estimate this value. The Markov Chain advances by proposing a new state $\Psi'$ compared to the old state $\Psi$ in the joint topology, branch length and substitution model parameter space, and accept the new state with the probability $R$.

$$R = \min \left[ 1, \frac{f(X|\Psi')}{f(X|\Psi)} \times \frac{f(\Psi')}{f(\Psi)} \times \frac{f(\psi'|\psi)}{f(\psi')} \right]$$

With a typically flat prior assigning equal prior probability to every possible topology, branch length and substitution model parameter (within reasonable limits), the prior ratio will be 1. Likewise the proposal mechanism of the chain can be designed so that the proposal ratio will be 1. If so, the new state will be accepted with a probability that equals the ratio of the likelihood of the new state to the likelihood of the old state. Since we have now at each state specific values of the branch lengths and substitution model parameters, calculations are straightforward without integrals but, assuming independence among sites, include only the product of the likelihood of observing the pattern at each site in the alignment

$$f(X|T, v, \theta) = \prod_{i=1}^{c} f(x_i|T, v, \theta),$$

where $c$ is the total number of sites and $x_i$ is the pattern at site $i$. The function to calculate the likelihood for each site $f(x_i|T, v, \theta)$ depends on the model chosen (HKY85, F81, JC69, GTR, SYM, etc. with or without I and 1) but is identical to standard likelihood calculations and is basically a summary of the likelihood of all different ways states can be assigned to the internal nodes. Thus the likelihood ratio can be calculated rapidly to derive the acceptance probability $R$ and, back to the Markov Chain, if the new state has a higher likelihood than the old state, the step will always be taken, whilst if the new state has a lower likelihood the step will be taken with decreasing probability the lower the likelihood ratio. The joint parameter space will be sampled in proportion to the posterior probability of the parameters and each can be extracted by sampling the chain so that e.g. the posterior probability of tree topologies is equal to the fraction of time each topology occurred in the sample. The value with the highest posterior probability of the branch lengths and substitution model parameters is simply the mean of all values from the sample. The chain generally starts at a random place in the parameter space, and a burn-in phase of the chain needs to be excluded before the summation of results since only after a while does the chain start to sample trees and parameter values in proportion to their posterior probability. How much to discard as a burn-in can be visually inspected in a chainstep-to-likelihood graph where an initial increase is followed by a stationary phase.
Results and discussion: summary of papers

_Paper I_

Within species variation in number and size of male suction cups turned out to be much higher than previously reported, providing the essential foundation for sexual selective forces. Furthermore, a simple uniform size component had no explanatory power since the variation in number of suction cups was not linked to variation in bodysize. _Graphoderus zonatus verrucifer_ was sampled across eight populations (lakes) and the estimated frequency of the granulate female morph varied from 0.06 to 0.87.

![Graph](image1)

A PLS-analysis revealed a significant correlation between this frequency and various aspects of male tarsi and suction cups (Fig. 6). The first component explained 91% of the variation in male variables and 76.5% of the female variation. What could be read from the variable loadings was that as the frequency of the granulate female morph increased, the male protarsal palette increased in size as did the three largest basalmost suction cups, while the smaller cups on both pro- and mesotarsal segments got smaller and more numerous (Fig. 7).

![Diagram](image2)

**Figure 6.** Correlation between frequency of female morphs and male tarsal characteristics. Scores plot on first component from PLS-analysis.

**Figure 7.** Illustration of the transformation along the first component (mesotarsi not shown), from high frequency of the smooth female morph (upper row) to a high proportion of the granulate female morph (lower row). Differences in males were most pronounced in number of small suction cups, size of three basal largest cups, and size of entire protarsal palette.
**Paper II**

The taxonomic revision recognised 7 nearctic and 6 palearctic species of *Acilius*, none of which occurred in both regions. A new species was described from the heavily populated northeastern USA (Fig. 8), a species that had previously been mixed up with *A. fraternus* in particular, but also *A. semisulcatus*. The new species occurs mostly in vernal ponds in deciduous forests, shaded and with plenty of dead organic matters like dark leaves and with or without vegetation. The type locality was fixed to Seth State Forest, (N38°45’W76°02’) a reserve outside Easton, Talbot Co., Maryland, USA. It was found here co-occurring with *A. fraternus, Dytiscus carolinus, D. verticalis, Hydaticus himarginatus, Thermonectus basillaris, Rhantus calidus, Hoperius planatus, Agabetes acuctus, Matus ovatus and Copelatus glyphicus*.

![Figure 8 Distribution in North America, female habitus and male genitalia of the new *Acilius* species described in paper II. Open symbols refer to state records (Wisconsin & Illinois) without further locality data whilst filled circles are within states identified localities.](image)

Lectotypes were designated for thirteen names, several of which had mixed type series and hence had been variously and confusingly interpreted in the past. Two previously recognised species names were synonymised with *A. abbreviatus*, the western nearctic species. This species is polymorphic both with regards to ventral colouration and in terms of the length of female sulci. Three females from coastal California had sulci merely present as sparsely haired shallow impressions towards the elytral apex. In addition the species hybridizes with *A. semisulcatus* where they
meet diagonally through British Columbia and the southwestern corner of Alberta. This zone is a previously recognised “suture-zone” or hybridizing zone of several other species pairs, where the transcontinental boreal biota meets the northern extension of the western mountain chain biota (Remington, 1968). Several species are severely threatened of global extinction. *Acilius kishii* represents a recent speciation and is confined to a single mountain lake in Honshu, Japan. This lake is well protected, but illegal introduction of fish into the lake could for example rapidly extinguish the species. *Acilius sinensis* seems to be known only from material described in 1915 and 1925. Known only from Yunnan and Sichuan in China, areas that have since been heavily disturbed by human development and activities, there is a real chance that the species is today globally extinct. The China water beetle survey (Jäch and Li, 1995-2003) has not recovered the species and my search at one of the type localities in Yunnan 2000 failed to recover the species. *Acilius duvergeri* seems to be declining and is considered extinct from the Dax, Landes area in France (Franck Baemul pers. comm.), where it was originally found and from where most museum specimens originate. A few modern localities are known in Spain, Portugal and Sardinia, but *A. duvergeri* is everywhere very rare and usually only single individuals have been found. The status in Morocco is unknown. It is on the IUCN global redlist as VU (Vulnerable).

**Paper III**

The fallacy today known as long-branch attraction (LBA) (Fig. 9) stems back to Felsenstein (1978) showing analytically that parsimony and compatibility methods were susceptible when evolutionary rates differed among lineages. Hendy and Penny (1989) used the expression “long edges attract” as they showed that the same thing can occur even with equal rates, but with unequal branch lengths.

![Figure 9 Long-branch attraction. Left side the true tree (A C) (B D). Methods can infer the wrong tree to the right (A B) (C D) because convergences along the two long terminal branches A and B overwhelms the true signal along the short internode X.](image)

More severely, if the conditions are right, the erroneous inference will be made with even greater certainty as more data of the same kind are sampled, a situation known as statistical inconsistency. The phenomenon has been in the core centre of the debate between parsimony advocates and likelihood proponents since the methods differ in sensitivity to the pitfall (Hillis et al. 1994a,b; Huelsenbeck, 1995, 1997, ...
1998; Huelsenbeck and Hillis, 1993; Huelsenbeck & Lander, 2003; Pol & Siddall, 2001; Siddall, 1998; Siddall & Whiting, 1999; Swofford et al., 2001). Despite the long history, the large body of literature accumulated over the years and its important ramifications for phylogenetic methodology, no review existed on the topic. Further there was a need to once and for all kill and bury the claim still expressed in recent student textbooks in systematics (Schuh, 2000), that LBA might be completely hypothetical and no examples based on real data existed. The major findings of the review paper can be summarized as follows; LBA is a very common phenomenon in real data. Although simulation studies are very important and informative, they are dependent of initial choice of model space and each should be taken with great caution. LBA is not restricted to parsimony inference or to simple substitution models but is a constant potential pitfall even with the most modern complex models available. Outgroups are always potential attractors of long-branched ingroup taxa and is the single most common cause of LBA among examples suggested to date. This should come as no surprise as already Hendy and Penny (1989), Wheeler (1990) and more recently Holland et al. (2003) have pointed out the conditions under which outgroups introduce LBA-artefacts. Enormous amount of data (e.g. complete genomes) is not a safeguard against LBA, when taxon sampling is poor. Increased taxon sampling and sampling more disparate kinds of data are probably the best available methods to avoid LBA. Long-branch extraction (incl. rooted and unrooted, ingroup-only, trees), separate partition analysis, methodological disconcordance (parsimony vs model-based), split graphs and parametric simulations (incl. random outgroups) are available methods for detecting LBA. Special importance of morphological data stems from the initially lower probability of suffering from LBA-artefacts as compared to molecular data. The greatest immediate need for the future is to evaluate different outgroup sampling schemes to reduce the risk of introducing LBA with the addition of outgroup taxa.

**Paper IV**
Bayesian analysis of three genes (Cytochrome Oxidase I, Histone 3 and Wingless) together with the morphological character matrix gave a fully resolved, complete taxon sampling phylogeny with clade support values ranging from 0.94 to 1.00 (Fig. 10). The entire topology received a posterior probability of 0.85, and the four best topologies constituted a 95% credible set of trees. Such a well supported phylogenetic result is rare, but provides an unusually robust backbone upon which evolutionary scenarios can be tested. The greatest strength is that the taxonomic sampling is complete, i.e. all known extant species are included. Probably more than 99% of all phylogenetic studies are to a greater or lesser degree biased by incomplete taxon sampling. Incomplete taxon samplings are normally not random but biased towards common, widespread, easy to collect species. Since rarity can have both biological and phylogenetic causes, such bias can potentially confound various tests of evolution or adaptation, when based on the phylogeny.
Figure 10. Phylogeny of Acilius from Bayesian analysis, posterior probability of topology = 0.85. Branch lengths estimated from 3:rd codon positions of the COI gene. Scale bar = 0.1 expected number of substitutions per site.

Character optimization corroborated the hypothesis that female dorsal surfaces and male tarsal suction cups are involved in an antagonistically coevolving arms race. Major inferred character transformations coincide perfectly between the two sexes on identical internodes (hypothetical ancestors). This is the first time coevolutionary intersexual arms race has been shown where uncertainty in phylogenetic reconstruction has been taken into account and taxon sampling is complete.

**Conclusion and future studies**

Diving beetles probably provide the most elaborate morphological example of an antagonistic arms race between the sexes. As such it has a great potential to become a very important model system of studies on sexual conflict and arms race. In particular the existence of species with two co-occurring female morphs provides an
exceptional natural opportunity to test and experiment with the effect of female counteradaptations without experimental removal of the trait. This thesis has used comparative and phylogenetic approaches to investigate the correlation of the male and female traits. What is badly needed now is more experimental work quantifying important aspects of the mating system like mating frequency, female cost of mating where actually drowning or at least oxygen deprivation can turn out to be important, the degree of assortative mating, if any, in the species with dimorphic females etc. Also the underlying genetical basis and inheritance of the traits need to be investigated, e.g. are the sulcate elytra of female *Dytiscus* regulated from a single locus, is the sulcate condition inherited via a dominant or recessive allele etc. The strict dimorphism in the system (i.e. normally no intermediates exist between smooth and sulcate females) makes genetical studies particularly suitable. The physical mechanics of the male suction cups also need further attention, especially how size and shape of suction cups affect adhesion ability on surfaces with varying degree of smoothness or structure. Finally, diving beetles might be very suitable for studies on the degree to which sexual conflict and arms race can lead to speciation as is the exciting recent focus in the field. Are the dimorphic species half-way towards speciation like envisioned in Gavrilets and Waxman’s (2002) model or is the mating system in fact stabilizing morph frequencies (Härdling and Bergsten, unpubl. manuscript)? The overview in paper III has widespread and important ramifications for the practical work of every systematist. That the artefact of long-branch attraction is so common in real dataset analyses, demands an awareness of the pitfall in every phylogenetic analyses performed, at least with molecular data. Identifying that outgroups are the most common cause of LBA (which I do not claim originality of) immediately presents a precautionary step that can be taken in every analyses, i.e. perform the analysis with and without the outgroups included! My claim that a combination of several of the LBA-detection methods suggested to date can be sufficient to corroborate LBA as the least falsified explanatory hypothesis of a phylogenetic outcome, should provide an alternative to the irrelevant, unscientific, verificationist claim, still encountered, that LBA has never been proven in real datasets.

Svensk sammanfattning

Dykarskalbaggar är rovdjur på andra vattenlevande insekter, kräftdjur, grod- och fiskyringel både som larver och vuxna. Artrikast är de i vegetationsrika dammar och sjöar, helst utan fisk, och i Sverige finns drygt 150 arter (i världen ca 3900) med en storleksvariation från 2mm till 4,5cm. Hanarna av dykarskalbaggar har som regel modifierade fram- och mellanfötter. De innersta tre fotlederna är breddade till en platta som är bestyckad med sugkoppar i olika storlek och antal. Sugkopparna använder hanen när han skall greppa en hona vid parningsförsök. Honorna har inga breddade fotter med sugkoppar men skiljer sig även från hannarna hos flera arter på att ryggen är grövre skulpterad på olika sätt. Det kan vara fåror, vårtigheter, hår eller ett nätverk av intrryckta linjer som finns på honans halsköld och täckvingar. Det har spekulerats mycket i vad funktionen är för dessa fåror och andra strukturer, då de är kraftiga och ögonenfallande. Till och med Darwin uppmärksammade dykarskalbaggarnas märkliga könsbundna morfologi och försökte inkorporera dem i sin teori om sexuell selektion. Han skrev att fårorna och håren på honans rygg var en
hjälp till hanen för att greppa honans slippriga kropp så att parningen skulle underlätta. Denna syn på samarbete mellan könen är dock idag förlegad, och istället har man uppmärksammat att det råder ständiga konflikter mellan könen. Konflikterna grundar sig i grund och botten på att honans investering i avkomman genom äggen är mycket större än hanens investering i avkomman genom spermier. Tillsammans med att själva parningen ofta medför avsevärd risker och kostnader har man visat att i många system så står könen under motsatt selektion vid möten, dvs. när en hane och hona möts lönar det sig för hanen att para sig, medans för honan så kostar det mer än hon tjarjar på det. Vid parningsbeteendestudier har det också visat sig att parning, eller försök till parning ofta inleds med en ”fight” där honan försöker bli av med eller kasta av den parningsvilliga hanen. Vi förväntar oss av detta att konflikten mellan könen skall leda till regelrätta kapprustningar för att öka kontrollen över parningsbesluten. Hanar selekteras för att övervinna honans kostnad motstånd med diverse utrustningar eller stimuli och honor selekteras för att motverka hanarnas försök att förera deras motstånd.

I denna avhandling har jag satt in dykarskalbaggarnas eleganta könsbundna karaktärer i ljuset av de nyligt utvecklade teorierna om sexuella konflikter och kapprustningar mellan könen. Urprungshypotesen som jag ville testa var om hanarnas sugkoppar och honornas ryggsskulptur är del av en evolutionär kapprustning mellan könen. Jag angriper hypotesen främst från ett fylogenetiskt (fylogeni=hypotes om det evolutionära släktkapet mellan organismer) perspektiv, eftersom kapprustning är en evolutionär process som spänner över stora tidsrymder. Med hjälp av en släktaskhypotes kan man se bakåt i tiden och på så sätt spåra en eventuell evolutionär process genom århundraden. I uppsats I jämför vi först olika populationer av en och samma art där honorna förekommer i två former (en slät och en med vårtig rygg) och testar om det finns något samband mellan proportionen av honliga former och hur hanarnas sugkoppar ser ut i populationen. Det visar sig att honformen proportionen och hanarnas sugkoppars storlek och antal är signifikant korrelerade över de åtta studerade populationerna (sjöar) i tre norrländska landskap. Detta är en prediktion av hypotesen om att de honliga och hanliga karaktärerna är del i en evolutionär kapprustning även om en korrelation inte bevisar den bakomliggande processen. Dessutom kvantifierar vi inomartssvariationen i antal sugkoppar hos ytterligare två arter dykarskalbaggar, vilken visar sig vara mycket stor och i ringa utsträckning kan förklaras med kroppstorleksvariation. Uppsats II är en taxonomisk revision av ett släkte dykarskalbaggar som sedan används för att testa kapprustningshypotesen i uppsats IV. En taxonomisk revision innebär att man rader ut hur många arter som finns beskrivna, vilka namn som skall användas, vilka namn som är synonymer till redan beskrivna arter, m m samt gör gruppen mer lättstuderad för kommande studenter genom beskrivningar, nycklar, illustrationer och utbredningskartor för alla arterna. För att reda ut vilket art olika namn refererar till krävs ofta att man identifierar typexemplar bevarade på museer, dvs den eller de individer som ursprungsförfattaren studerade och använde vid beskrivningen, vilket kan vara individer bevarade sedan Linnés tid och framåt. Totalt besökte 11 naturhistoriska museer i Europa, USA och Kanada och 8 ytterligare länades material av, vilket innebär att för revisionen studerades och databasfördes information om över 5000 individer (vilket blå låg till bas för utbredningskartorna). Revisionen kommer fram till att i släktet *Acilius* finns 34 olika giltiga namn som representerar 6 arter i palearktis (Europa, Nordafrika och Asien ung. norr om en linje längs Himalayas sydslutning ) och 7 arter i den nearktiska (USA och Kanada) regionen.
en välstödd släktskapshypotes undersöka hur de hanliga och honliga karaktärerna har utvecklats och om man kan se gemensamma mönster. Data till släktskapsanalysen utgörs av morfologiska karaktärer, en mitokondriell och två nukleära gener. För att ta fram dessa data har fältarbetet inneburit att samla in färskt material av alla arterna vid ett flertal expeditioner till Japan, Kina, Ryssland, Sydeuropa, Kanada och USA. Analysen som baseras på Bayesiansk statistik resulterar i en mycket välstödd släktskapshypotes där medelhavshotrovningen *A. duvergeri* är systerart till de resterande 12 arterna som i sin tur är uppdelade i en palearktisk och en nearktisk grupp. När man optimiserar hur punkturer, honornas håriga fåror och hanarnas sugkoppar har utvecklats på trädet så framgår direkt ett samevolutionärt mönster. Mönstret består av att stora förändringar i honornas och hanarnas karaktärer är koncentrerade kring identiska grenar i trädet (förfäder). Ryggpunktur sammanfaller med en första differentiering av hanarnas sugkoppar till tre stora och många små. Utvecklingen av håriga färnor på honans rygg sammanfaller i sin tur med en ytterligare mycket tydlig differentiering där en sugkopp blir mycket större än de andra, och de små blir mindre samtidigt som de femdubblas i antal. Dessutom sker en återgång i båda karaktärssystemen på exakt samma närförberedelse även i färornas sugkoppar återgår till färre små och mindre differentiering bland de tre stora. Ett endemiskt japanskt artpar tilldalar sig särskilt intresse då ena arten med en utbredning begränsade till en enda sjö, verkar vara en mycket nyttig arbritning som ger en ledträd till vilket kön som ändrar sig först. Honorna har i denna art också förlorat de håriga färorna, medan hanarnas sugkoppsdifferentiering är relativt oförändrad. Även om det bara är baserat på en observation, så kan i brist på annan bevisning denna ordning förutsättas vara hur de tre andra tre stora förändringarna i trädet också skedde; honorna ändrade sig först och hanarnas sugkoppsdifferentiering är responsen. Som syntes av avhandlingen förutspår dykarskalbaggar ha en mycket stor potential som ett modellsystem för studier av samevolutionära kapprustningar mellan könen och sexuella konflikter. Mycket experimentella undersökningar återstår att göra för att ta fram grunddata kring paringssystemet. Av särskilt intresse är de arter som har två olika former av honor, vilket dels är en utmaning att förklara inom ramen för sexuella konflikter och dels öppnar helt nya experimentella möjligheter.

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"Bugs are not going to inherit the earth – they own it now. So we might as well make peace with the landlord."
(Thomas Eisner, 1989)