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Description of Thermonectus nigrofasciatus and Rhantus binotatus (Coleoptera:Dytiscidae) mating behavior

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and form for publication:

Approved by the Thesis Committee:

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DESCRIPTION OF *THERMONECTUS NIGROFASCIATUS* AND *RHANTUS BINOTATUS* (COLEOPTERA: DYTISCIDAE) MATING BEHAVIOR

BY

LAUREN M. CLEAVALL

B.S., Biology, San Diego State University, 2005

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
Biology

The University of New Mexico
Albuquerque, New Mexico

August, 2009

DEDICATION

I would like to dedicate this manuscript to my mom, Kathy Cleavall, and to my dad, Bob Cleavall. You have been my biggest support system, my best friend, and my guiding light. You will always be my inspiration to achieve the world.

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I acknowledge Kelly Miller, my advisor who has taught me that "you learn by doing." Your faith in my ability to overcome obstacles and achieve my goals never diminished, even through the most trying times. I would not be where I am today if it wasn't for your guidance and support, as an advisor, as a teacher, and as a friend. I am truly grateful to have been a member of your lab.

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ABSTRACT

Models of sexual conflict predict that diving beetles should respond morphologically and behaviorally during mating events to overcome the opposing sex. Morphological and behavioral adaptations may result from pressures by one sex to overcome resistance to mating attempts of the opposite sex. Morphological data supports an evolutionary arms race in diving beetles (Dytiscidae) exhibited in a series of adaptations attributable to sexual conflict between males and females. Males of certain Dytiscinae have sucker shaped adhesion setae on their front and mid tarsi to improve attachment to the female's elytra prior to and during mating, whereas females have evolved grooves, ridges, and furrows in the elytra that appear to interfere with the adhesion of the male's suction cups before and during copulation.

Behaviors to overcome the opposing sex have been confirmed in *Dytiscus alaskanus*, but mating behavior in general has yet to be documented in other species of diving beetles. Behavioral data from more species will help clarify the evolution of sexual conflict in diving beetles. In this study, the mating behaviors of two species of diving beetles, *Thermonectus nigrofasciatus* and *Rhantus binotatus*, were described. *Thermonectus nigrofasciatus* and *Rhantus binotatus* mating behaviors were consistent with the model of sexual conflict in that males appear to coerce copulation on females and females resist male mating attempts. This study provides additional behavioral data that will enhance our understanding of the evolution of mating and sexual conflict in diving beetles.

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Introduction

Sexual conflict is the result of opposing optimal mating and reproductive strategies between males and females (Parker, 2006). In general, because of unequal investment in gametes, males increase their fitness through increased number of mates, whereas females increase their fitness by selecting fewer, higher-quality mates (Arnqvist & Rowe, 1995; Clutton-Brock & Parker, 1995; Parker, 2006). The diversity of mating partners, conflicting mating decisions, and differing objectives concerning gamete investment can make sexual conflict unavoidable, resulting in complex forms of sexual selection acting on both sexes (Chapman, 2006; Härdling & Smith, 2005; Mutanen, Kaitala & Monkkonen, 2006; Tregenza, Wedell & Chapman, 2006). Selection pressures on males and females to survive and reproduce can make sexual conflict a powerful force, especially when reproductive interests of the sexes differ and are in strong opposition, and often result in an evolutionary arms race (Hosken, 2005).

An evolutionary arms race is a struggle between males and females over multiple generations, in which both sexes evolve behavioral and morphological adaptations and counter-adaptations that assist in overcoming the opposing sex (Arnqvist, 1998; Chapman & Partridge, 1996; Mutanen & Monkkonen, 2006; Svensson et al.). Offensive adaptations by one sex are countered by defensive adaptations by the other sex in an asymmetric arms race (Dawkins & Krebs, 1979). Numerous traits can accumulate over evolutionary time which may result in a situation where offense-defense traits are maintained for multiple generations by both sexes (Härdling & Smith, 2005; Hardling & Bergsten, 2006).

In an arms race, adaptations are advantageous to the owner but are presumably disadvantageous to the other sex, resulting in an unequal balance of power between the sexes (Chapman, Arnqvist, Bangham, & Rowe, 2003; Härdling & Smith, 2005). Male traits evolve to give them an advantage in the decision to copulate but with costs to females, whereas females evolve traits that appear to resist or act against male mating attempts (Civetta & Singh, 2005; Cordero & Eberhard, 2003; Wigby & Chapman, 2004). Because males benefit by mating with multiple females, they may be selected to attempt to force copulation on females (Hardy, Ode & Siva-Jothy, 2005). However, females may experience increased costs through multiple copulations if mating restricts the female from feeding, resting, or predator avoidance. Exaggerated morphological adaptations (such as grasping devices in males and devices to interfere with male attempts to mate in females) and behavioral modifications (such as resistance behaviors to male mating attempts in females) could increase costs for both individuals, but the sex with the advantage may experience greater benefit than cost (Clutton- Brock & Parker, 1995).

An evolutionary arms race of behavioral and morphological modifications related to mating appears to be operating in the diving beetle subfamily Dytiscinae (Miller, 2003). Females in at least some species appear to actively resist male mating attempts in the form of rapid and erratic swimming behavior (Miller, 2003). In an apparent attempt to overcome the opposing sex before and during copulation, males of the clade Dytiscinae have evolved adhesive suction cups on their front and mid tarsi to improve attachment to the female's elytra (Bergsten & Toyra, 2001; Miller, 2003). Consistent with the model for escalating sexual conflict, females in several groups have independently evolved textured irregularities in the elytra which apparently interfere with the adhesion of the male's suction cups (Bergsten & Toyra, 2001; Bergsten & Miller, 2007; Miller, 2003).

Although critical in understanding sexual conflict in the Dytiscinae, knowledge documenting mating behavior is lacking in general. The only comprehensive description of diving beetle mating behavior is of *Dytiscus alaskanus* (Aiken, 1992). Additional study regarding the male's proficiency in forcing females to copulate, female behavioral resistance to mating, and female costs from prolonged mating duration would help determine to what extent sexual conflict is operating in diving beetles, and would form a foundation for study of the evolution of sexual conflict in general. Such a study of sexual conflict in diving beetles is inhibited, however, by lack of observations of mating behavior across the group. In an attempt to begin to remedy this, the objective of this study is to describe and compare the mating behaviors of *Thermonectus nigrofasciatus* and *Rhantus binotatus* to supplement the behavioral data on *Dytiscus alaskanus*.

Materials and Methods

(a) Specimens

Beetles were collected using an aquatic net and transported to the laboratory in plastic containers filled with pond water. *Thermonectus nigrofasciatus* specimens were collected on 9 Sept 2007 at Arizona, Yavapai Co., Tule Canyon Tank, 34°22.295'N 111° 51.252'W, K.B. Miller, colr. *Rhantus binotatus*, *Agabus*, and *Colymbetes exaratus* specimens were collected at New Mexico, Los Alamos Co., Valles Caldera National Preserve, 35°56.25N, 106°34.85W, 8,727′ elevation, K.B. Miller and L.M. Cleavall, colrs. *Rhantus binotatus* were collected on 8 Sep 2007 and 26 Sep 2007 and the other species were collected on 11 Oct 2008. Fifteen *T. nigrofasciatus* specimens, 20 *R.*

binotatus specimens, 10 *Agabus* specimens, and 10 *C. exaratus* specimens were collected. Specimens were identified by K.B. Miller (University of New Mexico) and voucher specimens were deposited in the Museum of Southwestern Biology Division of Arthropods, University of New Mexico (MSBA).

(b) Care of insects

Diving beetles were maintained in a 10 gallon freshwater aquarium and were fed freeze-dried crickets daily. Artificial rocks and plants were placed in the aquarium for beetles to grasp. Air temperature was kept constant at 21.11°C. Males and females were kept in the same aquarium, but a reinforced net was used as a partition to separate the sexes. The only time in which males and females were permitted to interact was during filmed observational sessions.

(c) Mating observations

Matings occurred under laboratory conditions. Male beetles were first observed in the 10 gallon aquarium. When a male displayed behaviors indicating an interest in copulation, the male was removed and placed into a separate mating tank. These behaviors included activities directed toward other males that were more typical of behaviors males direct toward females. After the male was positioned in the mating tank, a female was chosen at random and placed in the mating tank. Mating behaviors were observed, filmed, and notes were taken. When mating behaviors ceased, the pair was returned to the population of beetles in the aquarium. A vertical mesh net anchored from the base to the top of the aquarium was used as a partition to separate males and females.

(d) Recording of matings

For *Thermonectus nigrofasciatus* and *Rhantus binotatus* matings, a Sony Mini Digital Video Camera was used to document mating behaviors, date, and time. Videocassettes were digitally copied, archived using Windows Movie Maker, and duplicated onto a DVD for analysis. Approximately 1260 minutes of mating behavior was filmed.

(e) Observations of Other Species

Additional matings were observed for *Colymbetes exaratus*, *Agabus* sp., *Abagus gagates*, *Acilius mediatus*, *Acilius sylvanus*, and *Rhantus binotatus*. In general, these included fewer matings and observations were less complete or comprehensive than for *T. nigrofasciatus* and *R. binotatus*. Matings were filmed or notes were taken.

At the Valles Caldera National Preserve on 11 Oct 2008, an unidentified species of *Agabus* was observed mating and was examined in the field from 7 specimens. These matings occurred in a temporary holding container and were recorded. Following collection and for the next 48 hours, matings were documented between two pairs in the laboratory, but these matings occurred before specimens were separated by sex or could be filmed in the laboratory.

Colymbetes exaratus matings were filmed and observed under laboratory conditions between 1 Oct 2008 - 20 Feb 2009 and on 11 Sep 2007. These matings occurred in the mating tank but not all observations were filmed. When a male displayed behaviors indicating an interest in copulation (see above), the male was placed in the mating tank and a random female was placed in the tank. *Abagus gagates*, *Acilius* *mediatus*, *Acilius sylvanus*, and *Rhantus binotatus* mating observations were filmed under informal laboratory conditions between 2000-2001 by K.B. Miller.

(f) Description of Behaviors

Eight behavior patterns identified as mounting, lateral shake, male swimming, copulation, plug formation, probing, at surface, female breathing, and female swimming were used to describe *Dytiscus alaskanus* mating behaviors (Aiken, 1992). Initially, these behavior patterns were used as a guide when *Thermonectus nigrofasciatus* and *Rhantus binotatus* matings were first observed. However, considerable variation was seen in *T. nigrofasciatus* and *R. binotatus* matings compared to the more consistent behavior patterns identified in *D. alaskanus* matings (Aiken, 1992). General patterns were documented in all *T. nigrofasciatus* and *R. binotatus* matings, but complications arose when behaviors were placed into discrete categories. Some behaviors occurred intermittently, sporadically, or repeatedly and at least some variation was seen in every mating event.

To overcome these differences, behavior patterns were modified into phases and sub-phases that more accurately depicted *T. nigrofasciatus* and *R. binotatus* matings and allowed for variations to be clearly described among the general patterns. Three phases identified as 1) initiation of mating, 2) mating event, and 3) termination of mating categorized the consistent general patterns observed in all matings. Within these three phases, five sub-phases identified as A) male attachment, B) female resistance, C) copulation, D) post-copulatory guarding, and E) release, categorized the less consistent variations observed in all matings. Phases and sub-phases were specifically adjusted to more appropriately depict *T. nigrofasciatus* and *R. binotatus* behaviors, even though most of the initial eight behavior patterns identified by Aiken (1992) were observed.

Results

Mating behaviors for *Thermonectus nigrofasciatus* and *Rhantus binotatus* were categorized into three distinct phases identified as 1) initiation of mating, 2) mating event, and 3) termination of mating and five sub-phases identified as A) male attachment, B) female resistance, C) copulation, D) post-copulatory guarding, and E) release. Defined below, the three phases encompassing the five sub-phases provide the framework for descriptions in this study.

<u>Phase 1)</u> Initiation of mating occurs when a male attaches to the female as the female subsequently resists the male's attachment and tries to dislodge the mounted male. There appears to be no pre-copulatory courtship associated with initiation, and the female never initiates mating.

<u>Sub-phase A)</u> Male attachment occurs when a male approaches a female, grasps onto her with his suckers, and violently shakes her from side to side. The male places the prothoracic tarsi suction cups on the female's pronotum, and the mesothoracic tarsi suction cups around the female's mesothorax to the ventral surface (Figure 11). The male repeats attachment if the female resists and escapes or if another beetle disturbs the mating pair and causes a separation.

<u>Sub-phase B)</u> Female resistance occurs when the pair struggles against each other as the female tries to dislodge the mounted male. The female resists the male sporadically and irregularly following male attachment, during copulation, and intermittently throughout post-copulatory guarding. The duration, intensity, and number

of times the female resists the male varies depending on the situation and differs among females.

Phase 2) A mating event occurs when the male inserts the aedeagus into the apex of the female's abdomen, transfers a spermatophore, and guards the female after mating.

Sub-phase C) Copulation occurs as an intromission of the male aedeagus into the apex of the female's abdomen (Figure 1) in which the male transfers a spermatophore to the female (Figure 7). The number of times a male inserts the aedeagus into the apex of the female's abdomen ranges from 1-4 times and the duration lasts for a few seconds up to a couple of minutes. Intromission of the male aedeagus occurs anytime after the male attaches until the male releases the female. The male transfers one spermatophore to the female for successful copulation, even though the male inserts the aedeagus into the apex of the female's abdomen multiple times.

Sub-phase D) Post-copulatory guarding (referred to as guarding) occurs when the male remains attached to the female in a fixed immobile position. The male guards the female at or below the water's surface (Figures 5 & 19) where the pair's abdominal apices may or may not be exposed to atmospheric oxygen. Guarding duration and frequency varies even though the male guards the female following copulation but before release. Intervals of female resistance, episodes of abrupt lateral shaking of the female by the male, and a disturbance from another beetle commonly interrupts guarding. Typically, the male resumes guarding once the female ceases resisting and other conflicts subside. This pattern is repeated and occurs numerous times between the same mating pair.

Phase 3) Termination of mating occurs when the male releases the female or the female escapes the male's grasp through resistance behaviors.

Sub-phase E) Release occurs when the male shakes the female for 1-3 seconds and swims away. The male does not attempt to re-attach and appears to ignore the female. In some cases, the female resists and escapes the male's grasp.

Thermonectus nigrofasciatus

Phase 1) Initiation of Mating

A male initiates mating either immediately upon being placed together with a female or up to 90 minutes after this (Figure 2).

Sub-Phase A) Male Attachment

A male locates a female's position in the mating tank and quickly swims toward the female. Male attachment occurs when a female swims through the water, floats at the surface, or anchors to available substrate. A male first approaches a female, grasps her elytra with his suckers, probes the aedeagus at the apex of the female's abdomen, and violently shakes her from side to side. This sequence of behaviors occurs in 1-3 seconds (Table 1). A male attaches to the female before she swims away or escapes. A male repeats attachment if the female escapes, if the male resumes post-copulatory guarding following a separation from the female, or if another beetle disturbs the mating pair and causes a separation. The male places the prothoracic tarsi suction cups on the female's pronotum and the metathoracic tarsi suction cups around the female's mesothorax to the ventral surface (Figures 2 & 5).

In some cases, a male approached a female, grasped and shook her for 2-3 seconds, probed the aedeagus at the apex of the female's abdomen, and then released the female. This sequence occurred regardless of female resistance and, in these cases, was repeated until the male remained attached to the female.

Table 1. *Thermonectus nigrofasciatus* and *Rhantus binotatus* Sub-Phase Durations from September 2007 through December 2007

	Thermonectus nigrofasciatus	***Rhantus binotatus***
Dates Observed	21 Sep 2007 - 4 Dec 2007	24 Sep 2007 - 31 Oct 2007
Number of Observations	16	14
Attachment	1-3 seconds	1-3 seconds
Female Resistance	5-45 seconds	5-20 seconds
Copulation	1-10 minutes	1-10 minutes
Post-Copulatory Guarding	20-270 minutes	5-60 minutes
Release	1-3 seconds	1-3 seconds
Mating Event Duration	21-270 minutes	5-65 minutes

Table 1: Duration range displayed for each of the five *Thermonectus nigrofasciatus* and *Rhantus binotatus* sub-phases for a total of 16 and 14 observations, respectively. Sub-phases include male attachment, female resistance, copulation, post-copulatory guarding, and release from September 2007 through December 2007.

Sub-Phase B) Female Resistance

Female resistance occurs when the pair struggles against each other and the female tries to dislodge the mounted male. The pair moves rapidly in all directions, collides into the tank, and engages in rapid turning. The female erratically moves the prothoracic and mesothoracic legs and swims with the metathoracic legs. The male laterally shakes the female for 5-45 seconds (Table 1) until she ceases resisting or until she escapes. Typically, the male restrains the resisting female and proceeds with a mating event.

The female often resists the male following male attachment, periodically during copulation, and intermittently throughout post-copulatory guarding. The duration, intensity, and number of times the female resists the male varies depending on the female, the male, and the current situation. For example, the female vigorously resists the male when post-copulatory guarding persists for more than 60 minutes.

Phase 2) Mating Event

A mating event occurs after a male securely attaches to and restrains a resisting female. The male transfers a spermatophore to the female and post-copulatory guards the female.

Sub-Phase C) Copulation

Copulation occurs as a single intromission of the male aedeagus into the apex of the female's abdomen (Figures 3 & 18) in which the male transfers a spermatophore to a female. The pair moves very little during copulation. Copulation lasts for 1-10 minutes (Table 1) and occurs one time between a mating pair. The male probes the aedeagus at the apex of the female's abdomen before copulation, after copulation, or if the female begins to move. Male probing lasts for 2-10 seconds and a male repeats this behavior periodically. The male inserts the aedeagus into the apex of the female's abdomen multiple times before and after copulation.

Sub-Phase D) Post-Copulatory Guarding

Post-copulatory guarding occurs when the male remains attached to the female in a fixed immobile position. Typically, guarding occurs at the surface where water exposes the male and female's abdominal apices to the atmosphere (Figures 4, 5). Sometimes water covers both of their abdominal apices or only covers the female's abdominal apex and exposes the male's abdominal apex.

The female usually anchors to the side of the tank for structural support (Figure 20) with the prothoracic and mesothoracic tarsi or floats on the water's surface as the male guards her. The male places the prothoracic tarsi suction cups on the female's pronotum, and the mesothoracic tarsi suction cups around the female's mesothorax to the ventral surface (Figure 4). The male often adjusts his suckers apparently to gain a more secure attachment.

The male periodically flexes the apex of the abdomen up and down without touching the female. Flexing lasts for 2-10 seconds and occurs in every guarding sub-phase. The male flexes the apex of the abdomen when the female moves and then often shakes her from side to side. Less often, the female flexes her abdomen up and down which causes no reaction from the male. The male periodically sways gently from side to side which causes no reaction from the female.

Guarding duration and frequency varies for each mating pair. Intervals of female resistance, episodes of abrupt lateral shaking of the female by the male, and a disturbance from another beetle commonly interrupts guarding. The male usually resumes guarding once the female ceases resisting and other conflicts subside. This pattern is repeated and occurs numerous times between the same mating pair. Guarding lasts for 20-270 minutes (Table 1).

On 24 Oct 2007 and 26 Oct 2007, the *T. nigrofasciatus* male guarded the female for 270 and 210 minutes, respectively. Throughout this time, the female sporadically resisted the male, the pair struggled, and eventually the male restrained the female and continued to guard her. In both situations, the pair had adequate access to oxygen most of the time, and the female was not held below the water's surface.

Phase 3) Termination of Mating

Termination of mating occurred when the male released the female or the female escaped the male's grasp through resistance behaviors.

Sub-Phase E) Release

In the majority of cases, the male terminated post-copulatory guarding and released the female. The male abruptly shook the female for 1-3 seconds (Table 1) and separates from her, even if the female remains still during guarding. Once separated, the male brushes his front tarsi against his mouthparts in what appears to be a cleaning action. The male displays no attempts of re-attachment and appears to ignore the female following release.

In few cases, the female terminated guarding with resistance behaviors and escaped the male's grasp. The male sometimes reattached and resumed guarding if the female escaped. If the male resumed guarding, he eventually shook and released the female some time later.

Rhantus binotatus

Phase 1) Initiation of Mating

A male initiated mating either immediately upon being placed together with a female or up to 60 minutes after this.

Sub-Phase A) Male Attachment

A male locates a female's position in the mating tank and swims toward the female. Male attachment occurs when a female swims through the water, floats at the surface, or anchors to available substrate. A male first approaches a female, grasps her elytra with his suckers, and then shakes her from side to side. This sequence of behaviors lasts for 1-3 seconds (Table 1). A male attaches to the female before she swims away or escapes (Figure 6). A male repeats attachment if the female escapes, if the male resumes post-copulatory guarding following a separation from the female, or if another beetle disturbs the mating pair and causes a separation. The male places the prothoracic tarsi suction cups on the female's pronotum and the metathoracic tarsi suction cups around the female's mesothorax to the ventral surface (Figures 6 & 8).

In some cases, the male attached, shook the female for 2-3 seconds, released her without engaging in copulation, and eventually re-attached to the female. This occurred regardless of female resistance and was repeated 2-3 times until the male remained fixed to the female.

Sub-Phase B) Female Resistance

Female resistance occurs immediately after the male attaches as the female tries to dislodge the mounted male. The pair moves rapidly in all directions and engages in rapid turning as the pair tumbles through the water. The female frantically moves the prothoracic and mesothoracic legs and strikes at the male with the metathoracic legs. The male swims with the metathoracic legs and carries the female through the water. Female resistance lasts for 5-20 seconds (Table 1).

The duration and number of times the female resists the male varies depending on the female, the male, and the current situation. The female initially resists the male following male attachment but rarely resists the male during copulation or guarding. Typically, the male initiates the shaking and swimming movements during a mating event, even if the female appears steady and ceases resisting. The female resists the male following attachment, and the male responds by shaking the female from side to side. The male usually restrains the female and proceeds with a mating event.

Phase 2) Mating Event

A mating event occurs after the male securely attaches to and restrains the resisting female. The male transfers a spermatophore and post-copulatory guards the female.

Sub-Phase C) Copulation

Copulation occurs as an intromission of the male aedeagus into the apex of the female's abdomen (Figures 1& 7) in which the male transfers a spermatophore to the female. Typically, spermatophore material suspends from the apex of the female's abdomen during and after copulation (Figure 17), and the female maneuvers the spermatophore material with the metathoracic legs. During copulation, the male slowly bounces up and down, and the female kicks the metathoracic legs toward the male's aedeagus. Copulation lasts for 1-10 minutes (Table 1) and occurs one time between a mating pair. The male sometimes inserts the aedeagus into the apex of the female's abdomen multiple times before and after the male transfers the spermatophore.

In one situation, the female adjusted the suspended spermatophore material with the metathoracic legs, the spermatophore material fell off, and the female consumed the spermatophore material (Figure 9).

Sub-Phase D) Post-Copulatory Guarding

Post-copulatory guarding occurs when the male remains attached to the female in a fixed immobile position (Figure 8). Typically, guarding occurs below the water's surface where the water covers the male and female's abdominal apices. However, sometimes water exposes both of their abdominal apices or only covers the female's abdominal apex and exposes the male's abdominal apex. The female usually anchors to the base of the tank for structural support (Figure 19) with the prothoracic and mesothoracic tarsi as the male guards her. The male places the prothoracic tarsi suction cups on the female's pronotum and the metathoracic tarsi suction cups around the female's mesothorax to the ventral surface (Figures 6 & 8).

The male periodically shakes the female from side to side. The male brushes the metathoracic legs back toward the abdomen without touching the female. Throughout guarding, the male bounces up and down, twitches his body, and repeatedly opens and closes the apex of his abdomen as the female remains still. The female hardly moves and infrequently prompts a struggle between the pair. Guarding duration and frequency varies for each mating pair, but the male usually guards the female for 5 to 60 minutes (Table 1). In few cases, the pair separated if the male caused a disturbance and the female escaped or if another beetle interrupted the mating pair. The male often re-attached and resumed guarding.

Phase 3) Termination of Mating

Termination of mating occurred when the male released the female or the female escaped the male's grasp through resistance behaviors.

Sub-Phase E) Release

In most cases, the male laterally shakes the female for 1-3 seconds (Table 1) before he releases and separates from the female. Typically, the female remains still before the male shakes and releases her. Once separated, the male brushes his front tarsi against his mouthparts in what appears to be a cleaning action. Spermatophore material protrudes from the apex of the female's abdomen following release. The male does not attempt to re-attach and appears to ignore the female following release.

In a few cases, the male released, re-attached, resumed guarding, and again released the female until he eventually ignored her. The female rarely resisted and escaped to terminate guarding.

Observations of Other Species

With a few exceptions, the behaviors observed in *Abagus gagates*, *Colymbetes exaratus*, *Acilius mediatus*, *Acilius sylvanus*, and *Rhantus binotatus* mating events can be categorized into the three phases and five sub-phases defined above.

In *C. exaratus* matings, the male attaches to the female immediately following female placement in the mating tank. The male copulates 3-5 seconds after attachment with no indication of female resistance (Figure 11). The male remains attached to the female and swims in wide loops in a slow tumbling motion. The male guards the female at the surface and water exposes the pair's abdominal apices. Periodically, the female brushes the metathoracic legs back toward her abdomen and the male twitches the metathoracic legs. The male releases the female without attempts of reattaching, and spermatophore material suspends from the apex of the female's abdomen. A mating lasts between 4:00-7:00 minutes (Table 2).

In *A. gagates* matings, the male attaches with no female resistance (Figure 10), and the female swims through the water as the male remains attached. Throughout guarding and copulation, the male twitches and bounces up and down. The female brushes the metathoracic legs toward the male's abdomen and resists the male during guarding. The male releases the female and displays no attempts of reattachment. A mating lasts between 4:00-10:00 minutes (Table 2).

Table 2. *Agabus, Agabus gagates, Colymbetes exaratus, Acilius mediatus, Acilius sylvanus,* and *Rhantus binotatus* observation information

	Agabus	***Abagus gagates***	***Colymbetes exaratus***	***Acilius mediatus***	***Acilius sylvanus***	***Rhantus binotatus***
Dates Observed	11 Oct 2008 – 13 Oct 2008	2000-2001	1 Oct 2008 - 20 Feb 2009 & 11 Sep 2007	2000-2001	2000-2001	2000-2001
Number of Observations	5	2	17	3	1	2
Observation Method	Direct field observations	Filmed in laboratory	Filmed and observed in laboratory	Filmed in laboratory	Filmed in laboratory	Filmed in laboratory
Mating Event Duration	45 minutes	4:00-10:00 minutes	4:00-7:00 minutes	9:00-18:00 minutes	10:27 minutes	5:00-9:00 minutes

Table 2: Date observed, number of pairs observed, observation method, and total mating duration are displayed for *Agabus*, *Agabus gagates*, *Colymbetes exaratus*, *Acilius mediatus*, *Acilius sylvanus*, and *Rhantus binotatus* from 2000-2009.

In *A. mediatus* matings, the male attaches soon after female placement in the mating tank and the female resists attachment. The male copulates less than 60 seconds of attachment and probes the aedeagus at the apex of the female's abdomen. Throughout guarding, the male flexes the apex of the abdomen down, flutters the metathoracic legs for 3-15 seconds, and slowly bounces up and down. The male swims and carries the female to the base and to the surface of the tank (Figure 12). The male shakes and releases the calm female. In one case, spermatophore material protruded from the apex of the female's abdomen until a second beetle approached the female and consumed the spermatophore material. A mating lasts between 9:00-18:00 minutes (Table 2).

In *R. binotatus* matings (observed and filmed by K.B. Miller 2000-2001), the male attaches and shakes the female until she ceases resisting. Copulation occurs immediately following male attachment (Figure 14). The male guards the female at the surface and spermatophore material protrudes from the apex of the female's abdomen. In one case, the male released the female, the pair separated, the male reattached, the pair struggled, and the male resumed guarding the female. This sequence was repeated until the male eventually released and ignored the female. A mating lasts between 5:00-9:00 minutes (Table 2).

In one *A. sylvanus* mating, the male attached and shook the female until she ceased resisting. The male copulated 2 minutes following attachment (Figure 13). The male flutterd the metathoracic legs for 5-15 seconds and probed the aedeagus at the apex of the female's abdomen during copulation and guarding. The female brushed the metathoracic legs back toward the male's abdomen. The female resisted and escaped the male to terminate guarding. This mating lasted for 10:27 minutes (Table 2).

In *Agabus* matings, the male attaches and shakes the female from side to side until the female ceases resisting. The male copulates, guards the female, and finally releases the female. In one observation, a male proceeded with mating behaviors for over 45 minutes, despite less than ideal conditions (turbulent environment, minimal oxygen, limited water, and lack of space).

Reaction to Interruption or Disturbance

An additional beetle occasionally interrupts or disturbs the mating pair. When a second beetle disturbs the pair, the *T. nigrofasciatus* male adjusts his suction cups in what appears to be a more secure grasp and proceeds with copulation or guarding (Figure 15). A third beetle rarely causes a struggle between the mating pair in which the *T. nigrofasciatus* or *R. binotatus* female escapes (Figure 16). The *R. binotatus* male proceeds with copulation or guarding and seldom terminates mating behaviors when a second beetle continually disturbs him. Similarly, the *A. gagates* and *A. sylvanus* male proceeds with mating and usually maintains control over the female when another beetle disturbs the mating pair.

On 26 Oct 2007, a second *T. nigrofasciatus* male mounted backwards on the elytra of the guarding male, shook, and then held the guarding male below the water's surface. This initially caused no reaction from the mating pair until the second male continuously disturbed the guarding male and a struggle resulted between the mating pair. In another situation, the *T. nigrofasciatus* female escaped the guarding male, and the guarding male attached to the elytra of a second *T. nigrofasciatus* male. Both males remained still for 10 minutes, and the female rested on the opposite side of the tank.

From Nov 2007 through Sep 2008, the *T. nigrofasciatus* males continuously attempted copulation with other males in the aquarium. A *T. nigrofasciatus* male approached a second *T. nigrofasciatus* male, mounted to the elytra for 2-3 seconds, probed the aedeagus downward at the apex of the second male's abdomen, and released the second male. The males repeated these behaviors exhaustively while the females remained separated from the males by the partition in the aquarium.

Discussion

Comparison of *Thermonectus nigrofasciatus* and *Rhantus binotatus* Matings

Male attachment, female resistance, copulation, post-copulatory guarding, and release were documented in *T. nigrofasciatus* and *R. binotatus* matings. Initial male attachment occurred immediately after female placement in the mating tank or more than 90 minutes, in *T. nigrofasciatus*, and more than 60 minutes, in *R. binotatus* after female placement. The *T. nigrofasciatus* and *R. binotatus* males approached, attached, and shook the females for 2-3 seconds before the females tried to escape. For both species, this phase was sometimes repeated until the male remained attached to the female and proceeded with copulation.

The *T. nigrofasciatus* and *R. binotatus* females resisted the male between 5-45 and 5-20 seconds, respectively. *Thermonectus nigrofasciatus* female resistance appeared to be more persistent, forceful, and strenuous than *R. binotatus* female resistance. The *T. nigrofasciatus* male remained attached, even though female resistance behaviors appeared to almost dislodge the male. The *R. binotatus* male shook the female, even when the female was calm, whereas the *T. nigrofasciatus* male shook the female if the female started to move. The *T. nigrofasciatus* female swam through the water with the male attached, whereas the *R. binotatus* male swam through the water carrying the female.

Copulation was more visible in *R. binotatus* matings than in *T. nigrofasciatus* matings (Figure 17). Spermatophore material frequently protruded from the apex of the *R. binotatus* female's abdomen during and after copulation, whereas spermatophore material was rarely visible in *T. nigrofasciatus* matings. Throughout copulation, the *T.*

nigrofasciatus male repeatedly probed the aedeagus at the apex of the female's abdomen, whereas the *R. binotatus* male slowly bounced up and down. For both species, the male inserted the aedeagus multiple times before and after the spermatophore was transferred (Figure 18).

Thermonectus nigrofasciatus males guarded the female longer (20-270 minutes) than *R. binotatus* males (5-60 minutes). Typically, the *R. binotatus* male guarded the female at the base of the tank, whereas the *T. nigrofasciatus* male guarded the female at the water's surface. The *T. nigrofasciatus* female anchored to the side of the tank or floated on the water's surface (Figure 20), whereas the *R. binotatus* female anchored to the base of the tank or floated on the water's surface (Figure 19). The *T. nigrofasciatus* male flexed the apex of the abdomen up and down for 5-10 seconds repeatedly and swayed from side to side. The *R. binotatus* male bounced up and down, twitched his body, and opened and closed the apex of the abdomen.

Typically, the *T. nigrofasciatus* and *R. binotatus* male terminated guarding and released the female. Both males shook the female for 1-3 seconds, even if the female was calm, and released the female. The *T. nigrofasciatus* female sometimes resisted and escaped the male, which was less common with the *R. binotatus* female. The *R. binotatus* female rarely prompted a struggle or tried to escape.

Comparison with *Dytiscus alaskanus* Matings

Similar to *D. alaskanus* matings, the *T. nigrofasciatus* and *R. binotatus* males attached to the female's elytra regardless of her location in the tank and without any sign of pre-copulatory courtship (Aiken, 1992). The *D. alaskanus* lateral shake to prevent the female from resisting or escaping was consistent among *T. nigrofasciatus* and *R.*

binotatus (Aiken, 1992). When at the surface, the *D. alaskanus, T. nigrofasciatus,* and *R. binotatus* males were able to breathe, probe, and shake the females (Aiken, 1992). However, a male sometimes keeps a female from accessing atmospheric oxygen for long durations during guarding (Aiken, 1992; Miller, 2003).

Dytiscus alaskanus and *T. nigrofasciatus* males repeatedly attempted to copulate with other males, and both terminated contact within 2 seconds of mounting (Aiken, 1992). The males were unable to identify the sex of the individual they mounted (Aiken, 1992). The *D. alaskanus* male formed a mating plug following copulation, whereas a mating plug was not formed by the *T. nigrofasciatus* or *R. binotatus* male (Aiken, 1992). The *T. nigrofasciatus* male probed the aedeagus at the apex of the female's abdomen, whereas the *D. alaskanus* male probed the aedeagus specifically to touch the mating plug (Aiken, 1992). The *D. alaskanus* female started swimming which marked the end of mating, whereas the *T. nigrofasciatus* male shook and released the female to terminate mating (Aiken, 1992).

Comparison of Other Matings

The *C. exaratus* and *A. mediatus* male attached immediately following female placement in the mating tank, whereas attachment was delayed in *A. gagates*, and *A. sylvanus* matings. In *A. mediatus* and *A. sylvanus* matings, female resistance followed initial male attachment, yet female resistance was absent following initial male attachment in *C. exaratus* and *A. gagates* matings. The *A. gagates* female resisted the male during guarding but not after attachment.

The *C. exaratus* male copulated immediately following initial attachment, while the *A. mediatus* and *A. sylvanus* male copulated 1-2 minutes following initial attachment

(Figures 22, 23). Spermatophore material protruded from the apex of the female's abdomen during and following copulation in *C. exaratus*, and *A. mediatus* matings. Throughout copulation and guarding, the *A. gagates* and *A. mediatus* male bounced up and down, whereas the *A. mediatus* male flexed the apex of the abdomen down toward the female's abdomen. The *A. mediatus* and *A. sylvanus* male fluttered the metathoracic legs for 3-15 seconds and probed at the apex of the female's abdomen. The *A. gagates* male's body twitched whereas the *C. exaratus* male's metathoracic legs twitched. The *A. gagates*, *C. exaratus*, and *A. sylvanus* female frequently brushed the metathoracic legs against the male's abdomen.

The *A. gagates* female swam through the water as the male remained attached, whereas the *C. exaratus* and *A. mediatus* male swam and carried the female through the water. The *C. exaratus* male swam in wide anterior-posterior loops in a slow tumbling motion which was uncharacteristic of any other observed mating.

The *A. gagates*, *C. exaratus*, and *A. mediatus* male released the female and displayed no signs of reattachment to terminate mating. However, the *A. sylvanus* female resisted and escaped the male to terminate mating. *Agabus gagates*, *C. exaratus*, *A. mediatus*, and *A. sylvanus* matings were all between 4-18 minutes. The *A. gagates*, and *A. sylvanus* males proceeded mating behaviors when other beetles disturbed the mating pair.

Phylogenetic Comparison between Taxa

A close phylogenetic relationship between the taxa described in this study may or may not predict similar mating behaviors (Diagram 1). For example, *T. nigrofasciatus* and *D. alaskanus* are from the subfamily Dytiscinae, but *D. alaskanus* formed a mating plug following copulation and *T. nigrofasciatus* did not. However, observed behaviors for these two species were similar even though there were a few differences (see above). The *D. alaskanus* and *T. nigrofasciatus* males repeatedly attempted to mount and copulate with other males.

Rhantus binotatus, *C. exaratus*, *Agabus* are in the subfamily Colymbetinae, but differ in mating behavior. *Rhantus binotatus* and *C. exaratus* are from the tribe Colymbetini and are closely related (Alarie, 1995), yet the *C. exaratus* female did not resist the male following attachment, guarding lasted for 2-5 minutes, and the male released the female within 5 minutes of attachment. The *R. binotatus* female resisted the male following attachment and guarding lasted for more than 5 minutes. The *Agabus* female resisted the male during guarding and guarding lasted for more than 5 minutes. The *A. gagates* female swam through the water as the male remained attached, whereas the *R. binotatus* male swam and carried the female through the water. The *C. exaratus* male swam in wide loops in a slow tumbling motion which was not observed in *R. binotatus* matings. Even though *R. binotatus* and *C. exaratus* are the most closely related taxa, there were several dissimilarities in their behaviors.

Conclusion

The evolution of Dytiscidae mating behavior is complicated in that sexual conflict and cryptic female choice may be operating simultaneously as part of an evolutionary arms race (Miller, 2003). Morphological evidence showing the single derivation of male suction cups and five independent derivations of female counter-adaptations to male devices confirms intersexual conflict over mating decisions (Bergsten & Toyra, 2001; Miller, 2003). The sexual conflict model predicts that male mating attempts would be less successful with modified females, and female resistance would be more successful with modified elytra (Miller, 2003).

Behavioral data indicate that, for the most part, males were successful in restraining and copulating with resistant females (but see below). These behaviors were consistent with the model of sexual conflict in that *T. nigrofasciatus* and *R. binotatus* males initiated, appeared to force copulation on females, and terminated matings (Parker, 1979). Males responded to resistant females by vigorously shaking the females from side to side and adjusting their suckers to the female's elytra for a more secure attachment. In the majority of cases in which copulation was absent, it was because the male did not initiate mating.

The *T. nigrofasciatus* and *R. binotatus* females successfully resisted and escaped male mating attempts. Females knocked the males against the sides of the tank, brushed against the male's aedeagus with the metathoracic legs, swam erratically in all directions, and engaged in rapid turning. In some cases, females were successful and dislodged the male. In laboratory conditions (a small observation tank) the male was almost always able to find the female again and continue with mating. However, in a natural setting with turbid water, plant material, etc., the female may be able to escape the male.

The most important result from this study is the establishment of additional detailed observations of mating behaviors for use in more thoroughly understanding the evolution of mating and sexual conflict in diving beetles. This study documents the presence of behaviors related to sexual conflict between males and females of *T. nigrofasciatus* and *R. binotatus*. With morphological and behavioral data on Dytiscidae, it is apparent adaptations and counter-adaptations are used to overcome the opposing sex in an evolutionary arms race.

Appendix A

Figures 1-23

Figure 1: The *R. binotatus* male inserts the aedeagus into the female's genitalia.

Figure 2: The *T. nigrofasciatus* male attaches to the female's elytra.

Figure 3: The *T. nigrofasciatus* male copulates with the female.

Figure 4: The *T. nigrofasciatus* male guards the female.

Figure 5: The *T. nigrofasciatus* male guards the female.

Figure 6: The *R. binotatus* male attaches to the female's elytra.

Figure 7: The *R. binotatus* male passes the spermatophore to the female's genitalia

Figure 8: The *R. binotatus* male guards the female.

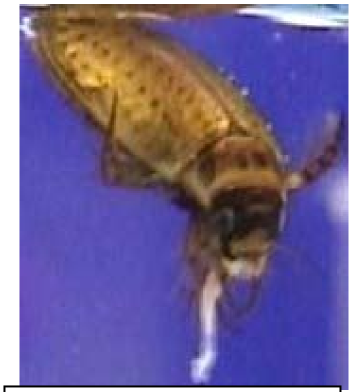

Figure 9: The *R. binotatus* female consumes free-floating spermatophore

Figure 10: The *A. gagates* attaches to the female.

Figure 11: The *C. exaratus* male inserts the aedeagus into the female's genitalia.

Figure 12: The *A. mediatus* male guards the female following copulation.

Figure 13: The *A. sylvanus* male copulates following attachment.

Figure 14: The *R. binotatus* male passes the spermatophore to the female.

Figure 15: A third *T. nigrofasciatus* male mounts to the elytra of the guarding male.

Figure 16: A third male *T. nigrofasciatus* agitates the guarding pair.

Figure 17: Sperm suspends from the *R. binotatus* female

Figure 18: The *T. nigrofasciatus* male copulates with the female.

Figure 19: The *R. binotatus* female anchors to the base of the tank.

Figure 20: The *T. nigrofasciatus* female anchors to the anchored

Figure 21: The *C. exaratus* guards the female.

Figure 22: The *A. sylvanus* copulates with the female.

Figure 23: The *A. mediatus* male copulates with the female.

Appendix B

Diagram 1

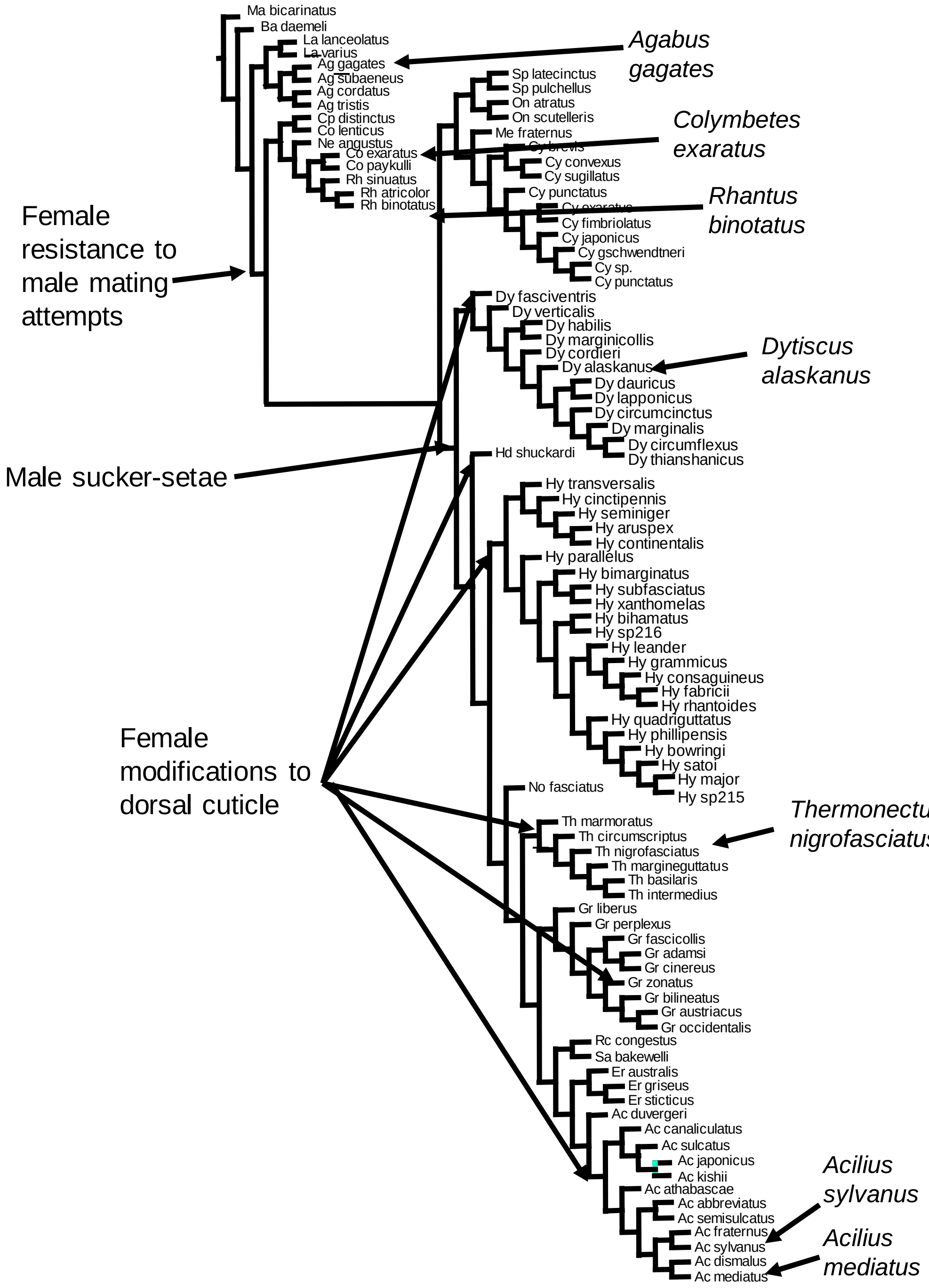

Diagram 1: *Acilius mediatus*, *Acilius sylvanus*, *Agabus gagates*, *Colymbetes exaratus*, *Dytiscus alaskanus*, *Rhantus binotatus*, and *Thermonectus nigrofasciatus* phylogeny with the derivation of female resistance to male mating attempts, male sucker-setae, and female modifications to dorsal surface noted.

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