

東海大學生命科學系

碩士論文

指導教授: 林仲平 博士

Dr. Lin, Chung-Ping

大就贏：雞冠細身赤鍬形蟲雄性競爭結果的決定因素及序列分析

To Win “Big”: Determining factors and sequential analyses in male-male
combats of a stag beetle, *Cyclommatus mniszehi* (Coleoptera: Lucanidae)

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摘要

同種的雄性時常會使用次級性徵像是武器來競爭配偶、領域和食物。瞭解打鬥機制和衝突解決因子對於解釋次級性徵的演化是很重要的。鍬形蟲科(Lucanidae)中的昆蟲擁有巨大且高度變異的大顎且常被使用於同種競爭中。我們利用序列分析(Sequential analyses)來檢視雞冠細身赤鍬形蟲(*Cyclommatus mniszehi*)的打鬥路徑和影響打鬥結果的因素。本研究結果指出雄蟲依循三條主要的打鬥路徑以及十一個不同的行為：啟始(I)、行走(W)、接觸(A)、對峙(E)、夾起對手(C)、抬起身體(B)、纏鬥(T)、下壓及推擠(Pr)、靜止不動(S)、追逐(U)以及撤退(R)。在對峙時(E)兩隻雄蟲會用大顎面對彼此，此行為後，雄蟲會直接將對手夾起並甩落木頭(第一路徑)。對峙後，雄蟲會用大顎下壓或推擠其他雄蟲(第二路徑)。當大顎和體型差異很小時，雄蟲之間的互動會進入劇烈打鬥，接著打鬥會持續直到一方被舉起或是撤退(第三路徑)。結果說明打贏的個體在劇烈打鬥中使用三種策略以擊退牠們的對手，而且雄蟲可能會於纏鬥(T)中透過大顎來評估對手的力量。打鬥時間與大顎以及體型的差異呈現負相關。大顎長度在決定鬥爭結果上較體型與家族來得更為重要。

關鍵字：次級性徵、同性性擇、武器、大顎、鍬形蟲、鍬形蟲科

Abstract

Males of conspecific species often used secondary sexual traits such as weapons to compete for mates, territories and food. Understanding the fighting mechanism and determinants of conflict resolution are important in explain the evolution of secondary sexual traits. Insects in the stag beetle family, Lucanidae, have enlarged and highly variable mandibles used for conspecific competition. We examined the fighting sequences of a stag beetle, *Cyclommatus mnischechi* using sequential analyses and the factors affecting fighting outcomes. Our study suggested that males of *C. mnischechi* followed eleven distinct behaviors and three main fighting routes. The behaviors included initiation (I), walk (W), approach (A), and encounter (E), clamp (C), body raising (B), tussle (T), pressure and push (Pr), stand still (S), pursue (U), and retreat (R). The two males stood head to head in encounter (E). After this behavior, males can either directly clamp opponents and throw them off the branch (first route), or use mandibles to pressure or push the opponent (second route). When mandible and body size differences between contestants were small, two males proceeded to escalated fights until one male was flipped up by the other male or one male retreated (third route). The results demonstrated that the winner used three strategies to defeat their opponents and the males may assess the strength of opponents through mandibles in tussling stage. The fighting duration was negatively correlated with differences of mandible and body size of the opponents. The mandible size was a more important factor in determining the fighting outcomes than the body size (elytra length) and families (genealogies).

Key words: Secondary sexual trait, intraspecific sexual selection, weapon, mandible, stag beetle, Lucanidae

Introduction

Sexual selection is considered as one of major evolutionary driving forces for creating an astonishing diversity of ornaments and weapons in animals (reviewed in Andersson, 1994; West-Eberhard, 1979). Rigid and sharp morphological outgrowths, like antlers, horns, tusks and spurs, are often protrude from the animal body, (Geist, 1966). These outgrowths can be used as weapons to deter predators (Siva-Jothy, 1987; Eberhard, 1982; Emlen, 2008) or as indicators for individual quality (Eberhard, 1982; Clutton-Brock, 1982; Moczek and Emlen, 2000). Animals used these morphological weapons to compete with conspecifics for limited resources, such as food, territory and mates (Andersson 1994; Moczek and Emlen, 2000; Judge and Bonanno, 2008). Actively engaging weapons in conspecific physical fights is an expensive way of resolving the conflict among individuals for acquiring limited resources (Siva-Jothy, 1987). Therefore, correctly gauging opponent's condition using ritual behaviors before "real" fights (Geist, 1966; Siva-Jothy, 1987) is a more effective alternative to minimize costs from fighting (Jakobsson et al., 1979; Small et al., 2009). Animals may perform behaviors to assess rival's quality or use specialized structure of their weapons to restrain the opponents (Emlen, 2008) and avoid physical damages (Moulds, 1977; Hongo, 2003; Okada and Miyatake, 2004; Emlen, 2008; Egge et al.,

2010).

In beetles, secondary sexual traits of males including enlarged horns and mandibles are weaponry outgrowths used for fighting (West-Eberhard, 1979; Andersson, 1994; Emlen and Philips, 2006; Emlen, 2008). These highly modified morphological weapons are often polymorphic within populations (Dominey, 1984; Andersson, 1994; Emlen, 2008). Larger males usually are equipped with fully developed weapons, while smaller males have only rudimentary forms of the same structures (Rasmussen, 1994; Emlen, 1997; Lai, 2001). Earlier studies indicate that males of different weapon morphs of a polymorphic beetle species adopt alternative mating strategies (Siva-Jothy, 1987; Emlen, 1997; Hongo, 2003; Egge et al., 2010). Males with larger weapons (majors) occupy territories and fight with intruding males to secure potential mates (Brown and Bartalon, 1986; Moczek and Emlen, 2000). In contrast, males possessing reduced weapons (minors) use satellite or sneaking behaviors to increase their mating opportunities (Siva-Jothy, 1987; Rasmussen, 1994; Emlen, 1997; Moczek and Emlen, 2000). As a result, phenotypic variations of these morphological weapons in beetles are directly linked to mating strategies.

Weapon size can sometimes honestly reflect the physical component of an individual's fighting ability (or resource holding potential; RHP) (Parker, 1974). Asymmetry of weapon size, motivation (resource value) and aggressiveness often

predict the outcomes of intraspecific escalated fights (Barlow et al., 1986).

Differences in size and its detailed morphologies of weapons (such as forks, grooves and denticles) might serve as cues for the assessment of rival's condition and strength in combats (Parker, 1974; Emlen, 2008). By interlocking weapons into positions, males can easily assess each other's strength without the risk of physical harm (Emlen, 2008). These ritualized and stereotyped fighting behaviors (interlocking, pushing and wrestling) have evolved in many ungulates (Geist, 1966) and several beetles (Siva-Jothy, 1987; Hongo, 2003) to facilitate these assessment functions (Emlen, 2008). Under the mutual assessment model (Enquist and Leimar, 1983; Enquist et al., 1990), pure-self assessment (Mesterton-Gibbons et al., 1996; Payne and Pagel 1996) and cumulative assessment (Payne, 1998), if the asymmetry in RHP of combatants is large, males would be expected to spend less time in these assessment behaviors for evaluating rivals. Therefore, the degree of RHP asymmetry between rivals and the duration of fights are expected to show a negative relationship.

Exaggerated mandibles have evolved in many lineages of stag beetles (Coleoptera: Lucanidae) (Emlen, 2008). These species exhibit enlarged and elaborate male mandibles which are highly diverse among species (Tetsuo and Shinji, 1994; Lai, 2001; Emlen, 2008). The weapon and body size show various scaling relationship or static allometry among species (Emlen and Nijhout, 2000). Males of polymorphic

species use these mandible weapons in combat with conspecific opponents over access to feeding sites such as tree saps visited by females (Tatsuta et al., 2001, Harvey and Gange, 2006, Kodric-Brown et al., 2006). Although detailed behavioral observations of male-male fighting were conducted on a few species (Mathieu, 1969; Hosoya and Araya, 2005; Shiokawa and Iwahashi, 2000; Hongo, 2005), very little is known concerning the behavioral sequences of fights, determinants of combat outcomes and the relationships between weapon, body sizes and contest duration for the majority of stag beetles. Earlier studies of the beetle's exaggerated male weapons and fighting behaviors focused on a horned beetle, *Allomyrina dichotomus* (Siva-Jothy, 1987; Hongo, 2003; Karino et al., 2005). The results indicated that the escalated fight begins with a series of behaviors before entering potentially damaging behaviors of close combats (Siva-Jothy, 1987). Males of *A. dichotomus* employ mutual appraising behavior ("shoving") for assessing rival's strength, in which males with smaller horns avoid the escalated fights with larger males (Hongo, 2003). The behavioral studies of two dung beetles, *Onthophagus acuminatus* and *O. taurus* demonstrated that males use two alternative mating strategies, in which large horned males defend entrances to tunnels while small hornless males encounter females by sneaking into tunnels (Emlen, 1997; Moczek and Emlen, 2000). No appraising behaviors were found during a typical male-male fight in *Onthophagus* dung beetles

(Emlen, 1997). In *A. dichotomus*, horn size appears to be the major factor determining the combat outcomes (Karino et al., 2005). Among *Onthophagus* dung beetles, both horn and body size are important in determining the fighting outcomes (Emlen, 1997).

In this study, we first characterized the behavioral interactions between rival males of *C. mniszehi* and examined the existence of ritualized fighting behaviors. Secondly, we investigated the relative importance of mandible size, body size, and family (genealogy) in determining the fighting sequences and outcomes. Finally, we examined the relationship between the size differences and contest duration and, the winner's size and contest duration, and the loser's size and contest duration.

Materials and methods

Study organism

Cyclommatus mniszehi (Thomsom, 1856) is a metallic brownish stag beetle inhabiting lowland forests of southeast China and northern Taiwan (Lai, 2001; Chang, 2006). It is the largest of three *Cyclommatus* species in Taiwan (Lai, 2001). The body length of *C. mniszehi* in males ranged from 28 to 58 mm in males and from 18 to 23 mm in females (Chang, 2006). Adults of this species were found feed on tree saps (e.g.

Cyclobalanopsis glauca, *Citrus reticulata* and *Fraxinus formosana* etc.) and their larvae feed on decaying tree trunks or branches buried beneath the ground. Males are equipped with a pair of enlarged mandibles which are variable in length, shape and the number of protruding teeth (Chang, 2006; Lai, 2001). The males of *C. mniszewi* can be divided into three major morphs (alpha, beta and gamma) according to the size and shape of mandibles (Lai, 2001; Kuan and Lin, unpublished) (Fig. 1). The mandibles of alpha males are equipped with two large denticles and have fork-shaped tips near the apex of mandibles. For alpha males, the length between the first and second denticles (a, Fig. 1) are shorter than that of the second and third denticles (b, Fig. 1). The beta male have a pair of small denticles near the base of mandibles, and the distance between the first and second denticles (a, Fig. 1) are longer than that of the second and third denticles (b, Fig. 1). Gamma males have no apparent mandibular denticles and the interior margin of the mandibles is scissor-like.

Beetle rearing

The larvae and adults of *C. mniszewi* were collected from four populations (WuLai, 24° 52' N, 121° 33' E; Sanxia, 24° 56' N, 121° 22' E; Shenkeng, 25° 0' N, 121° 37' E; Xindian, 24° 57' N, 121° 32' E) in northern Taiwan in May of 2007. The adults were

maintained in plastic containers (7.9 cm × 5.2 cm × 9.5 cm). The larvae were also maintained in plastic containers containing mixed wood chips from three plant species including common *Elaeocarpus* (*Elaeocarpus sylvestris*), Formosan Alder (*Alnus formosana*) and Formosan Sweet Gum (*Liquidambar formosana*) (Y-000, Bug's & I Beetle's Eco Exhibition, Taichung, Taiwan). These wood chips are the food for the larvae. The interior of plastic containers were sprayed with water every other days. The insects were kept in a laboratory with temperature at 22-25 °C and a day/night cycle of 12 hours. After emerged as adults, the beetles were fed with the commercial insect jelly (JB-001, Bug's & I Beetle's Eco Exhibition, Taichung, Taiwan). Before placing a mated female into a breeding container (31.5 cm × 20 cm × 13 cm), mating pairs were kept in separate mating containers (9.4 cm × 7.9 cm × 5.8 cm) for at least seven days to ensure copulation. The breeding containers were packed with wood chips (Y-000) of at least five cm deep and supplied with one or two pieces of the moist tree branch (*Elaeocarpus sylvestris* and *Liquidambar formosana*) for oviposition (Lai, 2001; Li, 2008). A small tree branch was placed on the surface of wood chips as grasping support for egg-laying females. Approximately one month after egg-laying period of the females, we collected these third instar larvae of the second generations and separately reared them to adults in the plastic containers. The males of the second and third generations were used in fighting experiments.

Fighting trials

Before setting up fighting trials, the daily activity of *C. mniszehi* adults was monitored using digital video cameras (HDR-XR200, Sony) for six days to determine appropriate time of the day for the contests. We randomly selected ten individuals (five males and five females) from different populations and placed each individual separately into a transparent plastic container (7.9 cm × 5.2 cm × 9.5 cm). We used white plastic sticks to make four quadrants on the cover of transparent plastic container. We recorded the number of the beetle's movement across the quadrants to represent the level of hourly activity of *C. mniszehi*. Fighting trials of male *C. mniszehi* were conducted on a modified cylindrical tree branch (*Acacia confusa*) of 20 cm in length and 2 cm in diameter. To increase the grasping power of the beetles, artificial scratches on the surface of the branch were made at 5 mm intervals along the long-axis using a ruler (Karino et al., 2005). The branch was then placed at the center of a transparent glass box (31.5 cm × 17.5 cm × 18 cm) 9 cm above the bottom of the box. Neither food nor females were provided during the contests to exclude the effect of resources on the outcomes of fights. Prior to each trials, we measured the full length of male's mandibles and elytra to the nearest of 0.01 mm using an electronic caliper (SV-03, E-BASE, Yunlin, Taiwan) as an indicator of weapon and body size,

respectively (Siva-Jothy, 1987; Setsuda et al., 1999; Hongo, 2003; Karino et al., 2005).

Mandible is a structure used in combat in stag beetles (Mathieu, 1969; Shiokawa and Iwahashi, 2000) and body size usually represents the individual's strength and ability to sustain injury (Archer, 1988). The trial males were randomly chosen from nine families (A-H and X). The males did not fight more than once on the same day and the same pair were not used as opponents on later trials. We marked a starting line 10 cm near each end of the branch with a black marker (MO-120-MC-BK, ZEBRA). The two trial males were simultaneously positioned at the line facing each other to control for the effect of prior residence (Hongo, 2003). Because *C. mniszzechi* are most active during the evening (see results), the fighting trials were conducted between 19:30 and 23:30 from July to August in 2009 and again from June to July in 2010. We recorded behavioral interactions of the trial males using digital video cameras. The fighting area was illuminated with a red light (3w, Acer) during the recordings to minimize the effect of lighting. The trial pairs that did not show any obvious fighting behavior were excluded from the analyses.

Ethogram and sequential analyses

We recognized twelve distinct behaviors in the male-male fights of *C. mniszzechi*

(Table 1). The fighting contest started when one male approached his opponent. The end of the contest was defined when one of the males flipped his opponent or when one male was pursued by his opponent (Table 1). We classified all behaviors into two types: low-intensity and high-intensity behaviors. Low-intensity behaviors are the behaviors without frequent physical contact between the opponents (≤ 0.33 contacts/s). High-intensity behaviors represent those stages with frequent physical contact between the opponents (≥ 6.33 contacts/s). All behaviors were mutually exclusive and only one behavior was scored at a given time. The behaviors were scored using Jwatcher (V.1.0, Blumstein and Daniel, 2007). All video recordings were scored twice independently, once for the winner and once for the loser. We used sequential analysis to examine the existence of nonrandom behavioral sequences exist in behavioral patterns (Egge et al., 2010). Before conducting the sequential analysis, we used a Markovian analysis to test the presence of dependencies between consecutive behaviors and to examine the structure of behavioral sequences (Blumstein and Daniel, 2007). The Markovian analysis in Jwatcher calculates the uncertainty statistics which provides an uncertainty value to predict the dependency between two successive behaviors (Bakeman and Gottman, 1997; Blumstein and Daniel, 2007). The uncertainty value corresponds to three different models. A zero-order model represents all behaviors in a sequence is independent. The first-order model assumes

the behavior is predicted entirely by prior behaviors. A second-order model indicated the next behavior is predicted entirely by two prior behaviors (Blumstein and Daniel, 2007). When the uncertainty value has a large decrease between two orders which suggesting the behavioral sequence approach Markov process at that order (Blumstein and Daniel, 2007). We recorded the duration of each behavior and counted the number of physical contact within each behavior. The sequential analyses produce the observed matrix, simple probability matrix and transitional probability matrix of the behavioral sequence. The observed matrix is simply the count of one behavior following another behavior in the sequence. The simple probability matrix calculates the probability of each behavioral sequence by the number of particular behavioral sequence divided by the total number of all behavioral sequences. The transitional matrix calculates the probability of each behavioral transition. The behavioral transition represents two successive behavioral events. These matrices were used to quantify the transitional frequency and to test the significance of two successive behavioral events following a stereotypical order (Blumstein and Daniel, 2007). The significance of the observed probability for a given behavioral sequence was tested using z-score ($p < 0.05$), therefore, any transition have z-score higher than 1.96 would be considered transition occur more often than expected by chance (Bakeman and Gottman, 1997; Blumstein and Daniel, 2007). We used Bonferroni correction for a

sample size of 19 ($\alpha < 0.0027$) to correct for inflated type I error due to multiple comparisons (Bakeman and Gottman, 1997).

Statistical analyses

The differences of mandible and body size between the winners and losers were examined using pair-*t* tests. Multiple logistic regression were used to analyze the importance of mandible size, body size and family for determining the fighting outcomes. Multiple logistic regression was conducted with the exclusion of males that fought more than once to exclude the effect of pseudo-replication. The outcome of the fights was used as the dependent variable (winner = 1; loser = 0) in multiple logistic regression. The difference in mandible and body size between the contestants and the families (family A to F) were used as independent variables. We excluded three families (family G, H and X) from the multiple logistic analysis regression because the low number (two) of contests in members of these three families. The goodness of fit for multiple logistic regression model was tested using likelihood ratio test statistics. A step-wise procedure based on AIC values was used to choose the most important variable in multiple logistic regression analyses (Venables and Ripley, 2002).

Multiple regression was used to test the correlation among fighting duration, mandible and body size of winners and losers. The winner's and loser's mandible size, and the winner's and loser's body size were used as independent variables. The fighting duration was used as a dependent variable. The fighting duration is counted from the encounter (E) stage to the end of fights. ANOVA was used to compare the mandible and body size differences between the two contestants for the four major fighting routes (route 1: clamp, males directly clamped his opponents and flipped them away; route 2: pressure and push, males used mandible pressured or pushed his opponents; route 3: tussle, males interlock their mandible and try to flipped each other; route 4: no fight). A post hoc analysis of Scheffe method was conducted to evaluate the pairwise difference among the four fighting routes. ANOVA was conducted to test whether differences in mandible and body size affected the choice of fighting routes. We used paired-T tests to examine whether the mandible and body size between the winners and losers of the fighting routes were different. R program (ver. 2.13.0, R development Core Team) was used to carry out multiple logistic regression. SPSS 12.0 (SPSS Inc, Chicago, Illinois) was used to carry out pair-*t* test, ANOVA and stepwise multiple regression.

Results

Activity Cycles

The numbers of movement for individual beetles were recorded for every two hours over six consecutive days (Fig. 2). The results demonstrated that *C. mniszewi* had a distinct day-night cycle, with increased movements during the evening from 6 PM to 6 AM and decreased activities from 6 AM to 6 PM during the day time hour. The activity pattern of males and females were similar and peaked after midnight.

Ethogram of Fighting Sequences in *C. mniszewi*

A Total of 201 trials of male-male fights were conducted. Among these trials, 95 (47%) of them resulted in fighting. In 28 (14%) trials, the contestants walked on the branch for an extensive period time and eventually did not result in fights. In 78 (39%) trials, one of the two males flew away from the fighting arena. The average duration of all contests resulting in fights lasted for 114 ± 162.35 seconds (range from 3.64 to 783.6s). Six distinct behaviors can be characterized in the contests (Fig. 3). They are encounter, body raising, tussle, clamp1, clamp2, pressure and push. After the encounter stage,

male-male interactions would follow three different sequences. The first fighting route was that one male directly clamped and flipped away their opponents after encounter (E). The second fighting route was when one male pressured or pushed his contestants into retreat. The third fighting route was when two males engaged in intensive wrestling. The fourth fighting route refers to no fights. Markovian analysis indicated fighting sequences in *C. mniszehi* belongs to first-order model (uncertainty value in zero-order model is 3.43; in first-order model is 0.9 and in second-order model is 0.822). The contest started when the two males were placed on the branch at the starting line ("Initiation", 10.32% of all behaviors). The male-male interactions (Fig. 4) began when one male walked toward his opponent ("Walk", 13.2%). After one male approached the other male ("Approach", 16.46%), the two males stood still with head facing each other ("Encounter", 15.59%). At this stage, they sometimes touch each other's antennas and mandibles (average 7 times \pm 5, n=95 pairs). After the encounter stage, the two males entered one of the three major behavioral routes of fighting sequences. In the first route, the winner male clamped their mandibles onto the loser's mandibles and raised them high up in the air ("Clamp", 7.22%), and then the loser were flipped over ("Flip", 7.44%) by the winner and the fights terminated. In the second route, after the encounter stage the winner used their mandibles to push or pressure ("Pressure", 1.79%) their opponents into retreat, then the winner either

pursued the losers or stood still. In the third route, after the encounter stage the two males gradually raised their bodies and mandibles against each other (“Body raising”, 7.28%). At this stage, the two males alternately raised their bodies and mandibles and the behavior was accompanied by rapid movement of antennas. Sometimes the mandible and antenna of the opponents touched each other constantly (average $3.64 \text{ times} \pm 1.94$, $n=69$ pairs). After reaching the highest position, the two males engaged in intense wrestling behavior which later developed into either prolonged escalated fights (“Tussle”, 6.9%) or entered the “Pressure” stage. At “Tussle” stage, the two males held each other’s body tightly with their mandibles interlocked and struggled to throw his opponent off the branch (“Clamp”, 7.22%) or forced him into retreat (“Retreat”, 1.58%). If one of the opponents retreated, the winners either stood at the same position (“Stand still”, 1.41%) or chased the loser (“Pursue”, 0.16%). The behavioral transitions occurred higher than expected by chance were used in the sequential analyses (Table 2). We divided all behavioral stages into low (without physical contact) and high (with physical contact) intensity behaviors (Fig. 4). There was no significant behavioral transition from the high intensity behaviors to low intensity behaviors except for non-conflict resolution behaviors leading to the end of fights, indicating no de-escalation in male-male fights of *C. mnischechi*, that is, no significant behavioral transition from high-intensity to low-intensity behaviors. The

most frequent low intensity behavioral transition is from “Walk” (W) to “Approach” (A) (93.39%). The transition from the encounter (E) to clamp (C) stage occurred with a frequency of 13.94% (Table 2). The transition from encounter (E) to body raising (B) had a transitional frequency of 46.69%, while the transition from encounter (E) to pressure (Pr) occurred with a lower frequency of 6.72%. The transition from body raising (B) to tussle (T) had a higher transitional probability (90.3%) than from body raising (B) to pressure and push (Pr, 6.72%). These results demonstrated that the contest resolution in male-male fights of *C. mnischechi* requires high intensity behaviors. The four behavioral transitions including (T) to (S), (T) to (Pu), (Pr) to (S) and (Pr) to (U) were unique to the winner, while the two transitions unique to the loser were from (T) to (R) and from (Pr) to (R) (Fig.5).

Effects of Mandible size, Body size, and Family on Fighting Outcomes

The results of the paired T-test indicated that males with larger mandibles won more fights than those with smaller mandibles ($t=5.723$, $n=95$ pairs, $p<0.0001$) (Fig. 6A).

The body size of winners was also significantly greater than that of losers (paired t -test, $t=4.689$, $n=95$ pairs, $p<0.0001$) (Fig. 6B). When mandible size is divided by the

body size, winner's value is significantly greater than that of losers (paired T-test, $t=4.701$, $n=95$ pairs, $p<0.0001$) (Fig. 6C). The scaling relationship of mandible and body size is different between the winner and loser, in which the winner have proportional larger mandible than the loser at a given body size (Fig. 7). The logistic regression analysis demonstrated that mandible difference between the two contestants is the most important factor in determining the fighting outcome (Table 3).

The Relationship between Size and Fighting Duration

The result of multiple regression analyses demonstrated a significant positive correlation between the loser's body size and the fighting duration (Table 4). The simple linear regression was used to test the significance of the relationship between the fighting duration and RHP differences (Table 5). The differences in mandible and body size between the two contestants revealed a significantly negative correlation with the fighting duration (Fig. 8; Table 5). The mandible and body size of the winner had no relationship with the fighting duration (Fig. 9A, B; Table 5). The fighting duration had significant positive correlation with mandible and body size of the loser (Fig. 9C, D; Table 5). The contest duration had a significant positive correlation with loser's mandible and body size in simple regression when considered winner's

mandible and body size as covariates in the multiple regression (Table 5). When loser's mandible size and mandible size difference were used as covariates in multiple regression, the result indicated showed that the fighting duration had no significant relationship with loser's mandible size or mandible size difference (Table 5). When loser's body size and absolute body size difference were used as covariates in the multiple regression, the result indicated that the fighting duration had no significant with loser's body size or body size difference (Table 5).

Trait Difference among the Four Fighting Routes

The differences in mandible size of the two contestants were not significantly different among the four fighting routes (clamp: Mean \pm SE = 2.144 \pm 2.637; pressure: 1.958 \pm 1.540; tussle: 1.460 \pm 1.025; no fight: 1.877 \pm 1.710, ANOVA: df = 121, F = 1.168, $p = 0.325$) (Fig. 10A). However, the body size differences between the two contestants were significantly different among the four fighting routes (clamp: Mean \pm SE = 1.273 \pm 1.05; pressure: 0.802 \pm 0.557; tussle: 0.629 \pm 0.529; no fight: 1.047 \pm 0.968, ANOVA: df = 121, F = 4.540, $p = 0.0005$) (Fig. 10B). The difference in body size of the two contestants for the fighting route 1 (Clamp) is significantly higher than those contestants in fighting route 3 (Tussle) (Scheffe's method, $p = 0.012$)

(Table 6). The two contestants taking the route 1 (Clamp) are significantly different in mandible ($t = 3.172$, $n = 22$, $p = 0.005$) and body size ($t = 3.585$, $n = 22$, $p = 0.002$). The contestants in the route 2 (pressure and push) are not significantly different in mandible ($t = 0.983$, $n = 15$, $p = 0.342$) and body size ($t = -0.282$, $n = 15$, $p = 0.782$). Both the mandible ($t = 5.647$, $n = 58$, $p < 0.0001$) and body size ($t = 4.366$, $n = 58$, $p < 0.0001$) differences are significantly different between two contestants in tussle (route 3). The contestants in the route 4 (no fight) are not significantly different in mandible ($t = -0.661$, $n = 28$, $p = 0.514$) and body size ($t = 0.161$, $n = 15$, $p = 0.873$).

Discussion

Our study demonstrated the detailed sequence of fighting behaviors in *C. mniszzechi* (Fig. 4). The behavioral transitions of conspecific fights in *C. mniszzechi* proceeded from low-intensity, no physical contact behaviors to frequent physical contact, high intensity behaviors (Fig. 4). Such transitional patterns suggested the existence of aggressively escalated fighting behaviors in stag beetles. Four behavioral transitions occurred after the initial encounter (E): walk (W), clamp (C), pressure or push (P) and body raising (B). Among these four behavioral transitions, (E) to (B) has the highest frequency (46.69%). The body raising stage probably is a prelude of tussle

(T) and only occurs in a conspecific competition such as the “shoving” behavior before the “pry” in horn beetles (Karino et al., 2005). The duration of (E) to (C) is the shorter than the (E) to (P), (E) to (B) and (E) to (W). In fights of *C. mniszewski*, we observed frequent physical contact in high intensity behaviors but no obvious injuries during the fights.

In *C. mniszewski*, the asymmetry of mandible and body size was negatively correlated with fighting duration. The winner’s mandible and body size revealed no significant correlation with the fighting duration. However, the loser’s mandible and body size had significant positive correlation with the fighting duration (Table 5; Fig. 9). Three game theory models, mutual assessment (Enquist and Leimar, 1983), cumulative assessment (Payne, 1998) and pure-self assessment (including ‘war of attrition without assessment’ and ‘energetic war of attrition’) (Mesterton-Gibbons et al., 1996; Payne and Pagel 1996; Arnott and Elwood, 2009), each has different predictions on the relationship between fighting duration and size of winners and losers. Both mutual assessment and cumulative assessment predict that the fighting duration is positively correlated with the loser’s size and negatively correlated with the winner’s size (Enquist and Leimar, 1983; Payne, 1998; Tylor and Elwood, 2003; Arnott and Elwood, 2009). The pure-self assessment predicts the fighting duration is positively correlated with the loser’s size, and slightly positively correlated or no

relationship with the winner's size (Mesterton-Gibbons et al., 1996). The contest duration is negative correlated with size differences for the three models (Tylor and Elwood, 2003; Arnott and Elwood, 2009). The results of multiple regression indicated that loser's body size determined the fighting duration (Table 6). Our results demonstrated that males of *C. mniszzechi* didn't actively assess their opponent's condition and their conflict resolution likely follow pure-self assessment.

Mandible (or horn) and body size is one of the important determinants in the outcomes of male-male contests in beetles (Siva-Jothy, 1987; Rasmussen, 1994; Moczek and Emlen, 2000; Hosoya and Araya, 2005; Karino et al., 2005). The results of multiple logistic regression (Table 4) clearly demonstrated that the mandible size is the most important factor determining the outcomes of male-male fights in *C. mniszzechi*. Based on our observations, the mandible was the first body structure to have physical contact between the opponents. The mandible size is considered as an indicator of strength in beetles (Geist, 1966; Karino et al., 2005; Emlen, 2008). The shapes of mandibles in beetles are highly variable with numerous protrusions (Tetsuo and Shinji, 1994; Lai, 2001). When animals inter-locked their weapons (e.g. horn or mandible) into certain positions, they may effectively assess the physical condition of each other without serious physical harm (Emlen, 2008). Although the result of multiple logistic regression indicated that mandible size was the most important factor

in determining the outcome of contests in *C. mniszehi*, the effect of the body size can not be completely excluded. Body size can reflect individual's strength and the ability to sustain injury.

In *C. mniszehi*, male-male interactions proceeded through four or five behavioral stages and followed three main contest sequences. In the first fighting route (Clamp), there was a significantly large difference in the mandible and body size of the opponents, and the larger males won the contest immediately by flipping their opponents. When size differences were large, males spent less time in gauging their opponents (Table 2, E→C). Winner males need strength to grab the barks and use their mandibles to lift and overturn their opponents. This strength came from adequate muscle mass within their bodies (Hongo, 2003; Karino et al., 2005). In the second fighting route (pressure), the mandible and body size between the winner and loser were not significantly different. The winner pressured and pushed its opponent into retreat. This behavioral sequence allows the beetles to resolve the conflict without cost of an escalated fight (pressure, the second route). In the third fighting route (tussle), the difference in mandible and body size between winner and loser was large. Comparing to the other two fighting routes (Table 2, E→C & E→Pr), the contestants displayed body raising (B) behaviors in which males spent more time in gauging their opponents for initiating an physical fight in third route (Table 2, E→B

& B→T). In body raising stage, both mandibles and antenna of opponents made frequent contacts. This is likely the stage in which the two males demonstrate their strengths and prepare to escalate into tussling. The tussling behavior represents a stage of assessing individual's strength because two rival beetles inter-locked their weapons (e.g. horn or mandible) into certain positions at this stage (Emlen, 2008). Males of *C. mniszehi* probably acquired more information on rival's condition during tussle.

Analyses of *C. mniszehi* suggested that the behavioral sequences of male-male competition are different between horn and stag beetles. Hongo (2003) studied the behavior involving in male-male competition of the horned beetle, *Allomyrina dichotoma*, and identified three main sequences with four behavioral stages. The outcomes of male-male fights in *A. dichotoma* were determined after the two contestants reaching the 'shoving' stage. *A. dichotoma* likely assesses the opponent's strength at this stage. In *A. dichotoma*, the loser males were chased or flipped by the winner males. For *C. mniszehi*, we identified four or five (start from Encounter) behavioral stages in three main fighting sequences. *C. mniszehi* has more complex behavioral structure than *A. dichotoma* for contest resolution. *C. mniszehi* can directly throw off its opponent at the first encounter without passing through all behavioral stages in the other two fighting routes. The winners of *C. mniszehi* can

also defeat their rivals by pressuring and pushing them off the arena. The details of fighting technique are also different between *C. mniszehi* and *A. dichotoma*. To flip off opponents, males of *A. dichotoma* insert their horns under opponents' bodies and then push and lift the opponents (Hongo, 2003). In contrast, males of *C. mniszehi* often position head to head with their rivals, and then engage their mandibles into interlocked positions and flip the opponents.

In this study, we described for the first time the behavioral sequences of aggression in male-male fights in *C. mniszehi* and identified the mandible size was the most important factor for determining outcomes of fights. Male *C. mniszehi* used three behavioral sequences to settle their contests. Our analyses demonstrated that the mandible was a reliable indicator for predicting fighting outcomes. The difference in mandible size, however, did not predict the fighting route used by the two opponents. The mandibles of *C. mniszehi* may be used as a tool to convey the quality of strength, but body size differences determined the behavioral route used by rival males.

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Table 1 Behaviors of male-male fights in *C. mniszewi*.

Behavior	Description
Initiation (I)	The two males were placed on the branch and faced to each other.
Walk (W)	Males walked toward its opponents.
Approach (A)	Males arrived at his opponent.
Encounter (E)	Males stood head to head and sometimes touched each other's mandibles and antennae.
Clamp (C)	One male clamped his mandibles onto the opponent's mandibles or body.
Body raising (B)	The body and mandibles of the two opponents raised against each other.
Pressure or push (Pr)	The mandibles of one male pressured and pushed the other male.
Tussle (T)	The mandibles of the opponents interlocked.
Stand still (S)	No physical movement of the two opponents
Pursue (Pu)	The male walked toward his opponent while he was moving away.
Retreat (R)	The male walked away from his opponent.
End	The male was flipped or chased by his opponent.

Table 2 Transitional frequencies between behavioral stages of fights in *C. mnischechi*

Behavioral transition	Frequency	Lasted time ^W (s)	Lasted time ^L (s)	Transition (%)	<i>z</i>	<i>p</i> -value
Significant transitions						
I → W	118	82.04±115.99	74.21±137.56	62.11	19.65	<i>p</i> <0.001
I → A	72	90.94±152.60	118±152.47	37.89	7.39	<i>p</i> <0.001
W → A	226	73.56±125.47	70.42±127.78	93.39	32.63	<i>p</i> <0.001
A → E	261	6.30±23.47	4.22±6.07	86.14	34.94	<i>p</i> <0.001
E → W	82	50.09±63.66	29.86±49.51	28.57	7.33	<i>p</i> <0.001
E → B	134	45.72±67.74	46.05±81.71	46.69	26.32	<i>p</i> <0.001
E → Pr(ed)	24	38.13±76.37	40.25±66.91	8.36	8.47	<i>p</i> <0.001
E → C(ed)	40	27.96±48.78	18.12±33.58	13.94	4.02	<i>p</i> <0.001
B → T	121	4.71±5.40	5.14±4.84	90.30	37.43	<i>p</i> <0.001
B → Pr(ed)	9	6.30±4.94	4.11±2.62	6.72	4.07	<i>p</i> <0.001
T → C(ed)	89	64.81±148.55	68.35±154.39	70.08	26.72	<i>p</i> <0.001
T → S	12	23.33±43.03	NA	9.45	7.42	<i>p</i> <0.001
T → U	2	147.60±155.75	NA	1.57	3.84	<i>p</i> <0.001
T → R	14	NA	35.56±71.45	11.02	8.27	<i>p</i> <0.001
T → M	4	380.26	88.65±138.80	3.15	5.43	<i>p</i> <0.001
C → F	132	24.78±90.13	22.85±88.58	99.25	39.64	<i>p</i> <0.001
Pr → R	15	NA	2.45±2.28	45.45	19.30	<i>p</i> <0.001
Pr → S	14	3.13±2.20	NA	42.42	19.03	<i>p</i> <0.001
Pr → U	1	6.98	NA	3.03	3.88	<i>p</i> <0.001
M → F	5	1.5±0.29	8.97±12.61	83.33	6.67	<i>p</i> <0.001
Non-significant transitions						
A → W	42	14.64±24.60	9.61±12.86	<i>13.86</i>	-0.44	<i>p</i> =0.661
W → E	15	41±64.52	38.46±27.02	6.20	-4.97	<i>p</i> <0.001
W → M	1	8.53	NA	<i>0.41</i>	0.13	<i>p</i> =0.890
E → A	5	NA	78.52±38.54	1.74	-8.00	<i>p</i> <0.001
E → T	1	NA	8.92	0.35	-5.14	<i>p</i> <0.001
E → M	1	14.1	NA	<i>0.35</i>	-0.05	<i>p</i> =0.963
B → C	4	3.69±2.80	NA	2.99	-2.25	<i>p</i> =0.024
C → E	1	NA	19	0.75	-5.28	<i>p</i> <0.001
T → E	6	88.43±137.41	78.35±121.09	4.72	-3.92	<i>p</i> <0.001
Pr → E	3	3.02±1.02	NA	<i>9.09</i>	-1.27	<i>p</i> =0.204
M → E	1	17.04	NA	<i>16.67</i>	-0.05	<i>p</i> =0.963
F → T	5	3.30±0.05	5.70±2.87	3.65	-1.85	<i>p</i> =0.063

The 0.05 probability level for all significant transitions; italics indicates is the transitions after Bonferroni-adjusted $\alpha < 0.0026$. W: winner; L: loser. I: initiation; A: approach; W: walk; E: encounter; B: body raising; T: tussle; C: clamp(ed); P: pressure (d) or push(ed); F: flipped; R: retreat; U: pursue; S: stand still; M: being clamped.

Table 3 Multiple logistic regression analyses using a dataset with the exclusion of males fighting more than once and a step-wise removal procedure.

Independent variables	B	SE	z-score	<i>p</i>
Full model				
dML	1.739	0.539	3.227	0.001**
dBL	-0.252	0.604	-0.418	0.676
Family B	1.088	1.175	0.925	0.355
Family C	0.162	1.49	0.108	0.914
Family D	1.518	1.274	1.191	0.233
Family E	1.117	1.775	0.629	0.529
Family F	0.407	0.938	0.434	0.664
Opponent B	-1.078	1.177	-0.915	0.360
Opponent C	-0.125	1.494	-0.084	0.933
Opponent D	-1.515	1.276	-1.188	0.235
Opponent E	-1.103	1.779	-0.62	0.535
Opponent F	-0.397	0.939	-0.422	0.673
Second model				
dML	1.544	0.481	3.209	0.001**
dBL	-0.206	0.57	-0.362	0.717
Family B	1.355	1.104	1.228	0.220
Family C	0.107	1.411	0.076	0.940
Family D	1.492	1.16	1.285	0.199
Family E	0.783	1.875	0.418	0.676
Family F	0.270	0.879	0.307	0.759
Third model				
dML	1.321	0.411	3.213	0.001**
dBL	-0.097	0.534	-0.182	0.855
Final model				
dML	1.269	0.287	4.425	0.0001***

B, regression coefficient; SE, standard error; dML, difference in mandible length; dBL, difference in body length

Table 4 Step-wise multiple regression analyse of the relationship between mandible size, body size and the fighting duration in *C. mniszehi*.

Independent variables	B	SE	z-score	p
Full model ($p=0.031$)				
Winner's ML	0.071	0.059	1.208	0.230
Winner's BL	-0.217	0.120	-1.811	0.074
Loser's ML	0.034	0.068	0.507	0.614
Loser's BL	0.130	0.137	0.949	0.345
Intercept	1.416	1.989	0.712	0.478
Second model($p=0.015$)				
Winner's ML	0.070	0.059	1.193	0.236
Winner's BL	-0.215	0.119	-1.807	0.074
Loser's BL	0.191	0.066	2.910	0.005**
Intercept	0.952	1.759	0.541	0.590
Third model($p=0.011$)				
Winner's BL	-0.115	0.085	-1.359	0.178
Loser's BL	0.196	0.066	2.969	0.004**
Intercept	0.370	1.694	0.218	0.828
Final model($p=0.007$)				
Loser's BL	0.179	0.065	2.752	0.007**
Intercept	-1.368	1.116	-1.226	0.223

B, regression coefficient; SE, standard error; ML, mandible length; BL, body length

Table 5 Regression analyses of the effect of mandible and body size on the fighting duration of *C. mniszehi*.

	b±SE	F	dfs	p
Simple regression analysis				
Loser's mandible size ($R^2=0.069$)	0.09±0.03	6.94	1, 93	0.010**
Winner's mandible size ($R^2=0.001$)	0.02±0.04	0.13	1, 93	0.724
Absolute size difference of mandible size ($R^2=0.101$)	-0.11±0.03	10.40	1, 93	0.002**
Loser's body size ($R^2=0.075$)	0.18±0.07	7.57	1, 93	0.007**
Winner's body size ($R^2=0.007$)	-0.07±0.09	0.62	1, 93	0.432
Absolute size difference of body size ($R^2=0.088$)	-0.23±0.08	9.02	1, 93	0.003**
Using either winner or loser's mandible size as covariates ($R^2=0.069$)				
Loser's mandible size as variable, winner's mandible size as covariates	0.09±0.03	6.73	1, 92	0.011**
Winner's mandible size as variable, loser's mandible size as covariates	0.0002±0.03	<0.0001	1, 92	0.996
Using either winner or loser's body size as covariates ($R^2=0.093$)				
Loser's body size as variable, winner's mandible size as covariates	0.20±0.07	8.81	1, 92	0.004**
Winner's body size as variable, loser's mandible size as covariates	-0.12±0.09	1.85	1, 92	0.177
Using either loser's mandible size or size difference of mandible as covariates ($R^2=0.102$)				
Loser's mandible size as variable, size difference of mandible as covariates	0.02±0.05	0.13	1, 92	0.723
Size difference of mandible as variable, loser's mandible size as covariates	-0.09±0.05	3.32	1, 92	0.072
Using either loser's body size and size difference of body as covariates ($R^2=0.099$)				
Loser's body size as variable, size difference of body as covariates	0.09±0.09	1.07	1, 92	0.304
Size difference of body as variable, loser's body size as covariates	-0.16±0.10	2.41	1, 92	0.124

Table 6 Multiple comparisons of mandible and body size differences between the two contestants among four different fighting routes using Scheffe's method.

Fighting route (I)	Compared route (J)	Mean differences (I-J)	SE	<i>p</i>	95% CI	
					Lower bound	Upper bound
Mandible size						
Clamp	Pressure	0.186	0.547	0.990	-1.367	1.738
	Tussle	0.683	0.409	0.429	-0.477	1.844
	No fight	0.267	0.466	0.954	-1.054	1.588
Pressure	Tussle	0.498	0.473	0.776	-0.845	1.841
	No fight	0.081	0.523	0.999	-1.402	1.565
Tussle	No fight	-0.416	0.376	0.747	-1.483	0.650
Body size						
Clamp	Pressure	0.470	0.254	0.336	-0.251	1.192
	Tussle	0.643	0.190	0.012*	0.104	1.183
	No fight	0.225	0.216	0.781	-0.389	0.839
Pressure	Tussle	0.173	0.220	0.892	-0.451	0.797
	No fight	-0.245	0.243	0.798	-0.935	0.445
Tussle	No fight	-0.418	0.175	0.133	-0.914	0.780

SE, standard error; CI, confident interval

Figure caption

Fig. 1 The three male morphs and a female of *C. mniszeechi*.

Fig. 2 The daily rhythm of *C. mniszeechi* in the laboratory. The vertical bars represent the average number of movement for every two hours in males (black, n = 5) and females (white, n = 5). Grey area indicates the night hour.

Fig. 3 Six distinct behavioral stage and the three fighting routes observed in male-male fights of *C. mniszeechi* (figures kindly drawn by Hsuan-Yu Peng).

Fig. 4 Flow chart indicates all behavioral transitions occurring in the contests of *C. mniszeechi*. The thickness of arrows represents the level of transitional probabilities. Box sizes and the associated numbers indicate the number of a given behavior. Dark and light grey indicate behaviors with and without physical contact, respectively.

Fig. 5 The Kinematic diagram of behavioral transitions in trial winners (A) and losers (B) of contests in *C. mniszeechi*. The thickness of arrows represents the level of transitional probabilities. Box sizes and the associated numbers indicate the number of a given behavior. Dark and light grey indicate behaviors with and without physical contact, respectively.

Fig. 6 The mandible size (A), body size (elytra length; B) and mandible size divided by body size (C) of the winner and loser males in *C. mniszeechi*. The horizontal line within each box indicates the median of the values. The box indicates 75 and 25 percentiles of the values. The vertical bars indicate the total range of the values.

Fig. 7 Scaling relationship of mandible and body size for the winners and losers.

Fig. 8 The relationships between the fighting duration and trait difference; in mandible size (A) and body size (B) of *C. mnischechi*.

Fig. 9 The relationships between the fighting duration and trait differences in the winner (A & B) and loser (C & D) of contests in *C. mnischechi*.

Fig. 10 The mandible size (A) and body size (B) differences among four different fighting routes in contests of *C. mnischechi*. NS, not significant; *, $p < 0.05$.

Figure 1

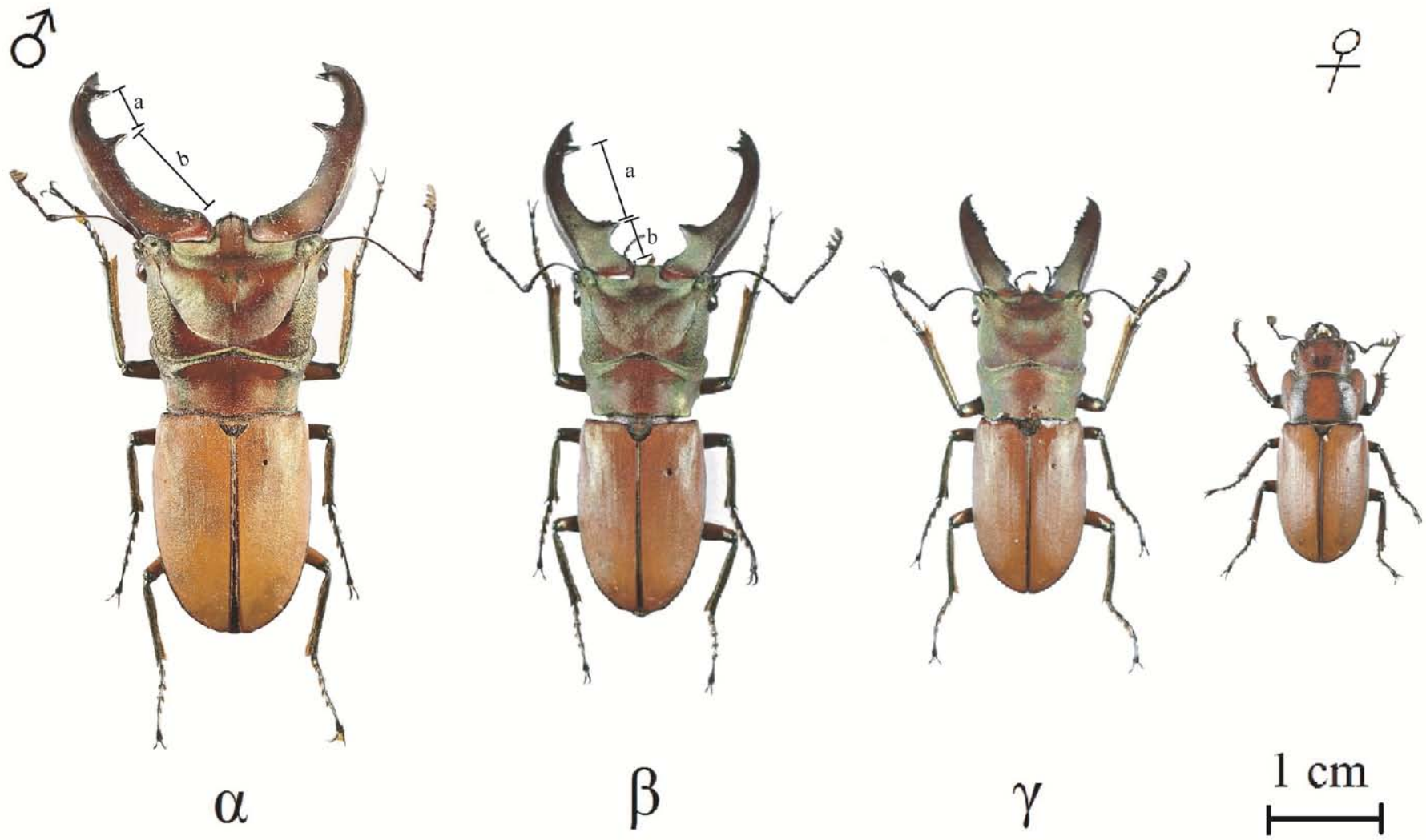


Figure 2

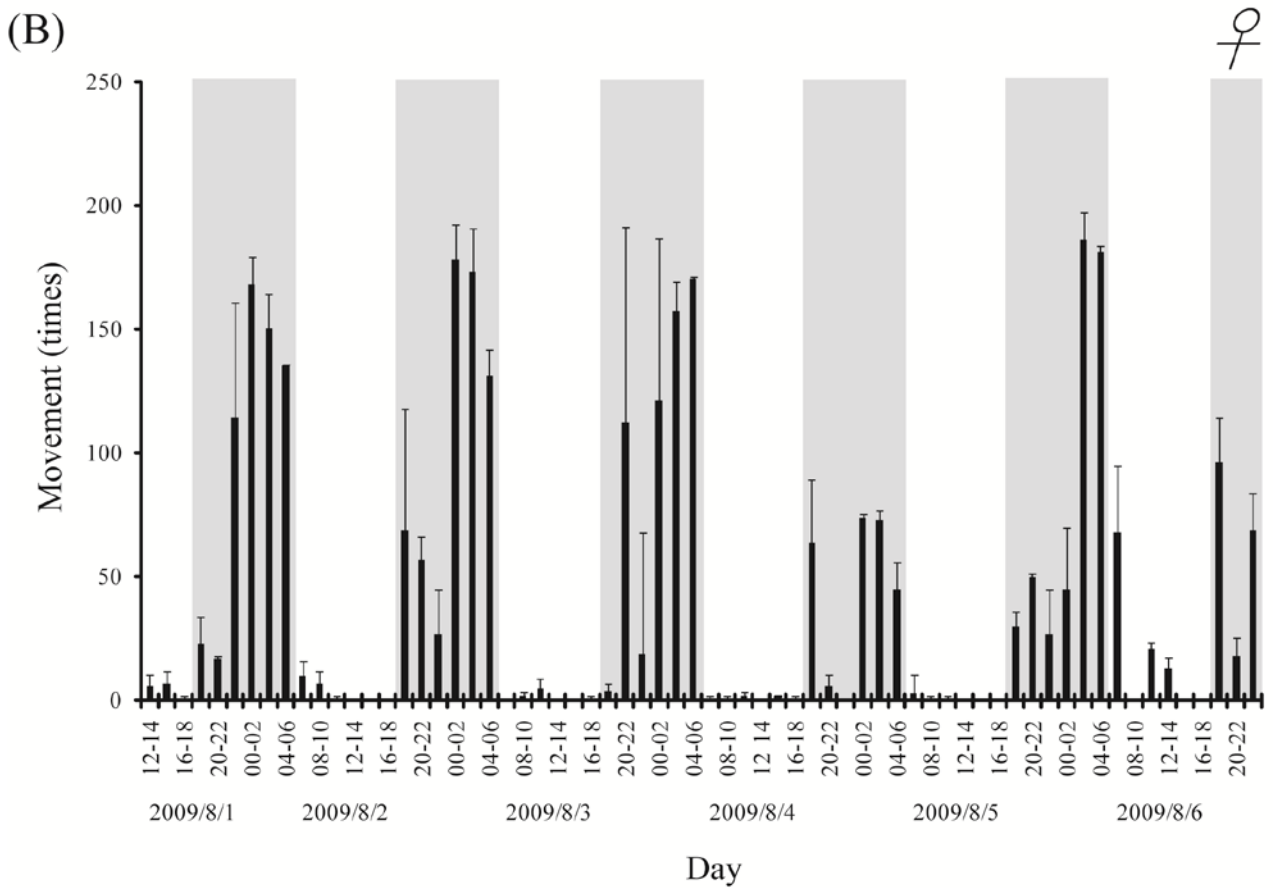
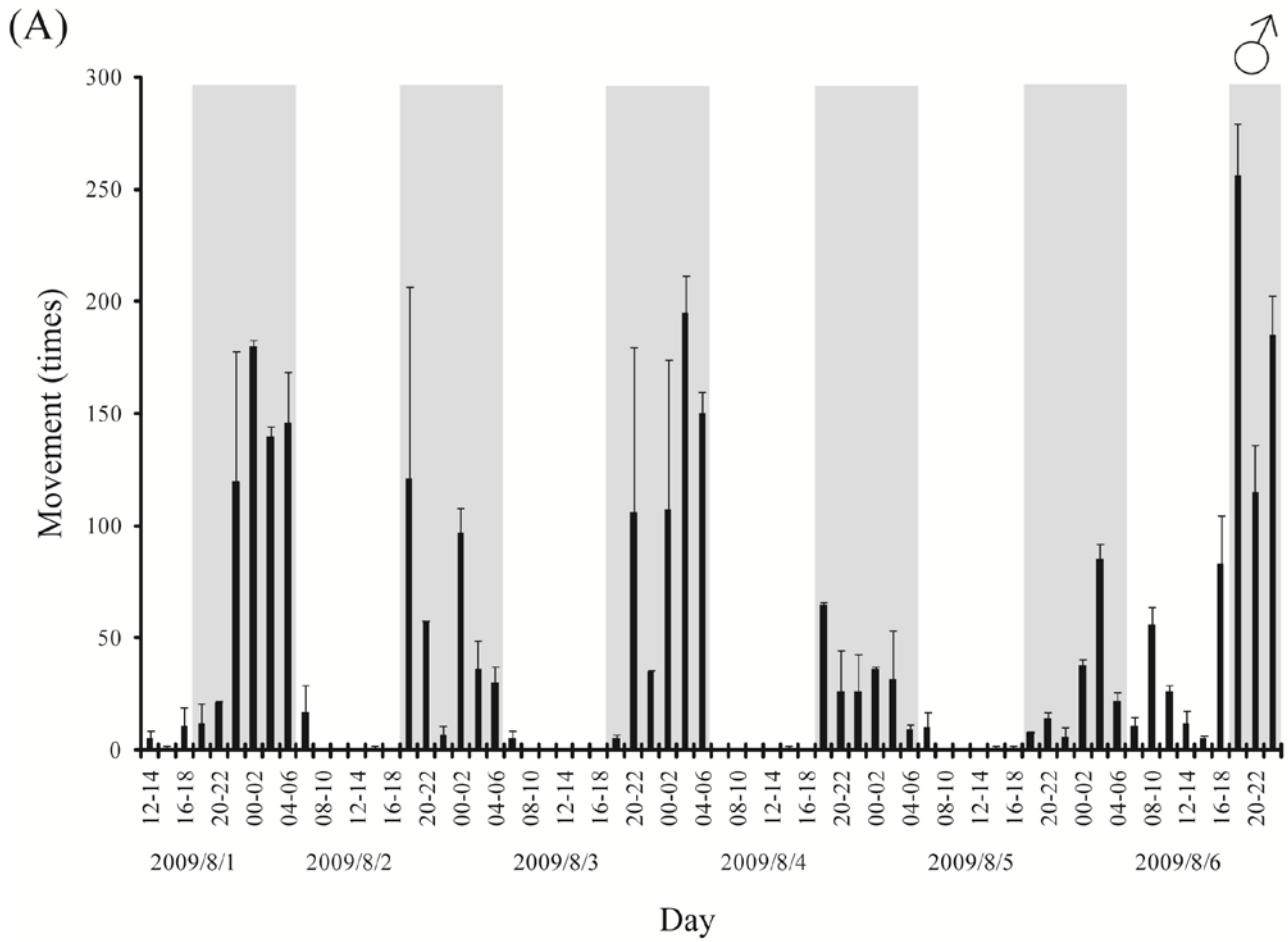


Figure 3

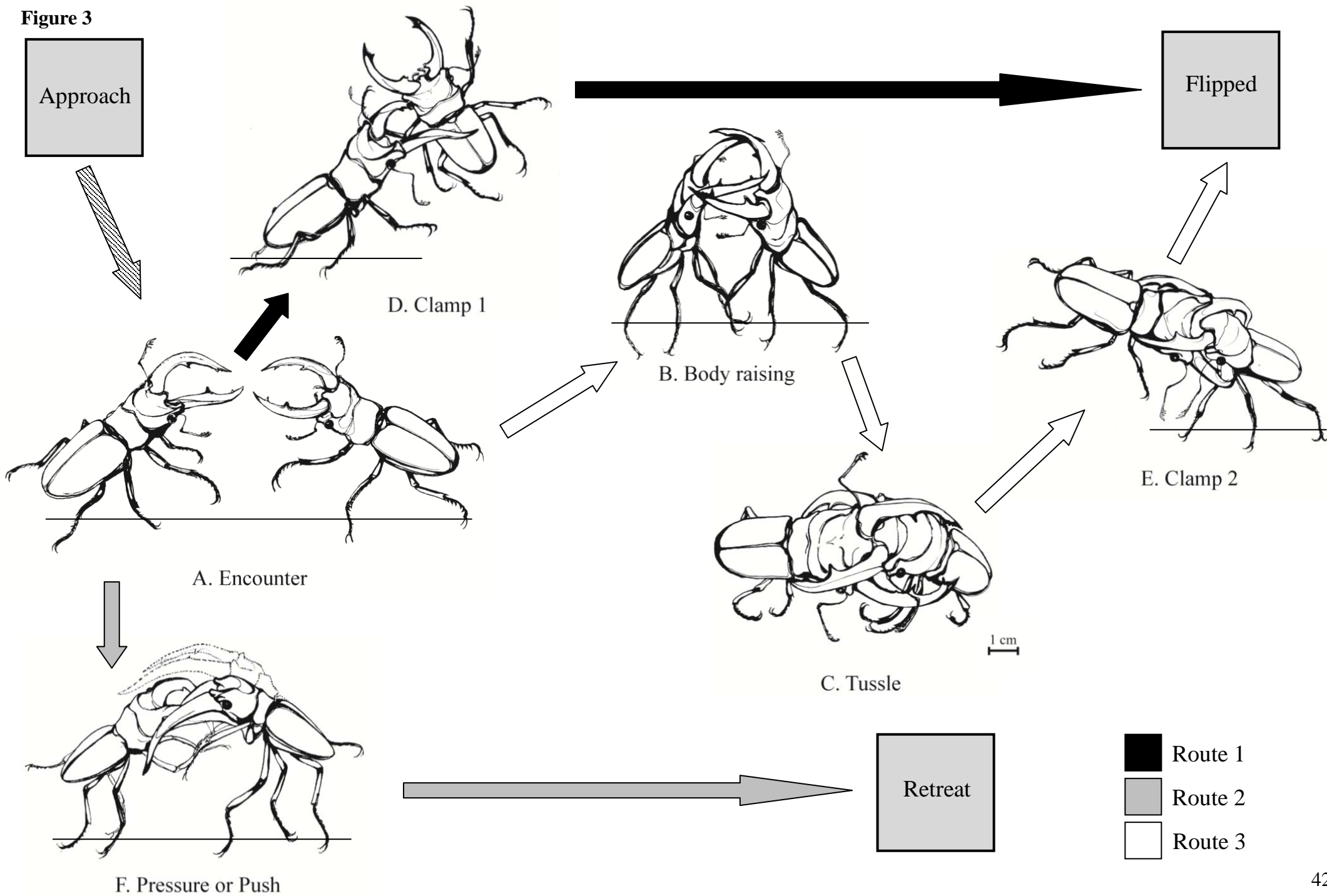


Figure 4

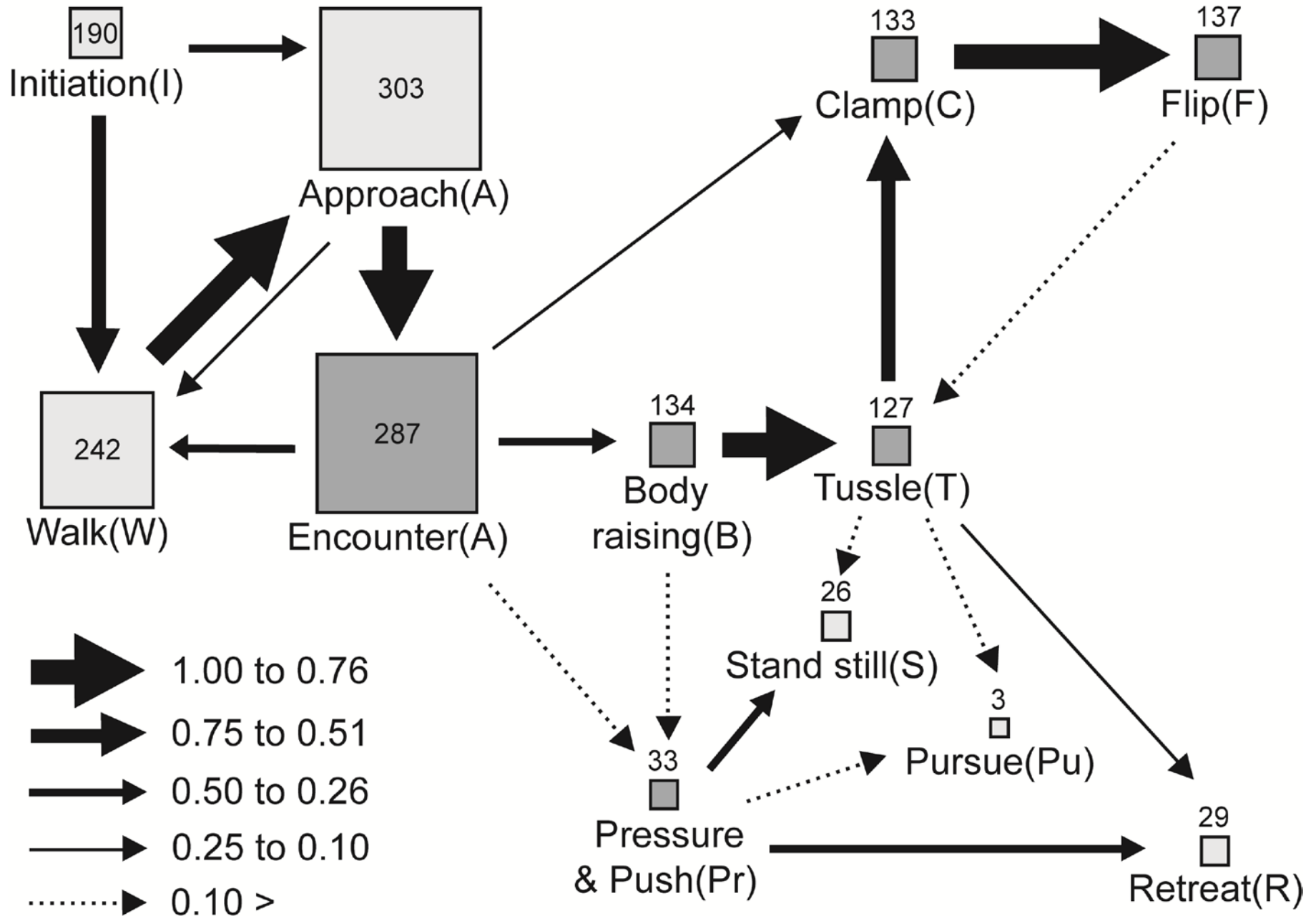


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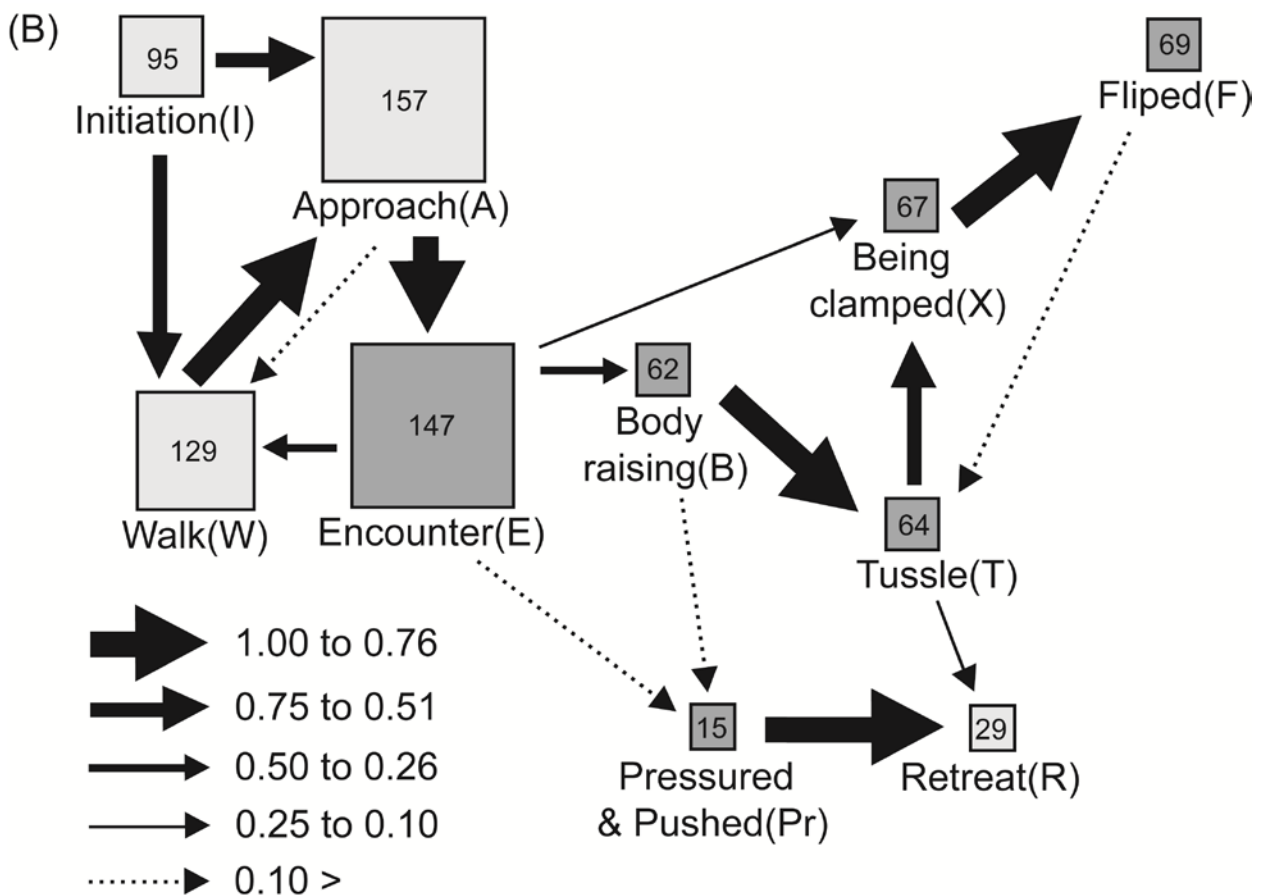
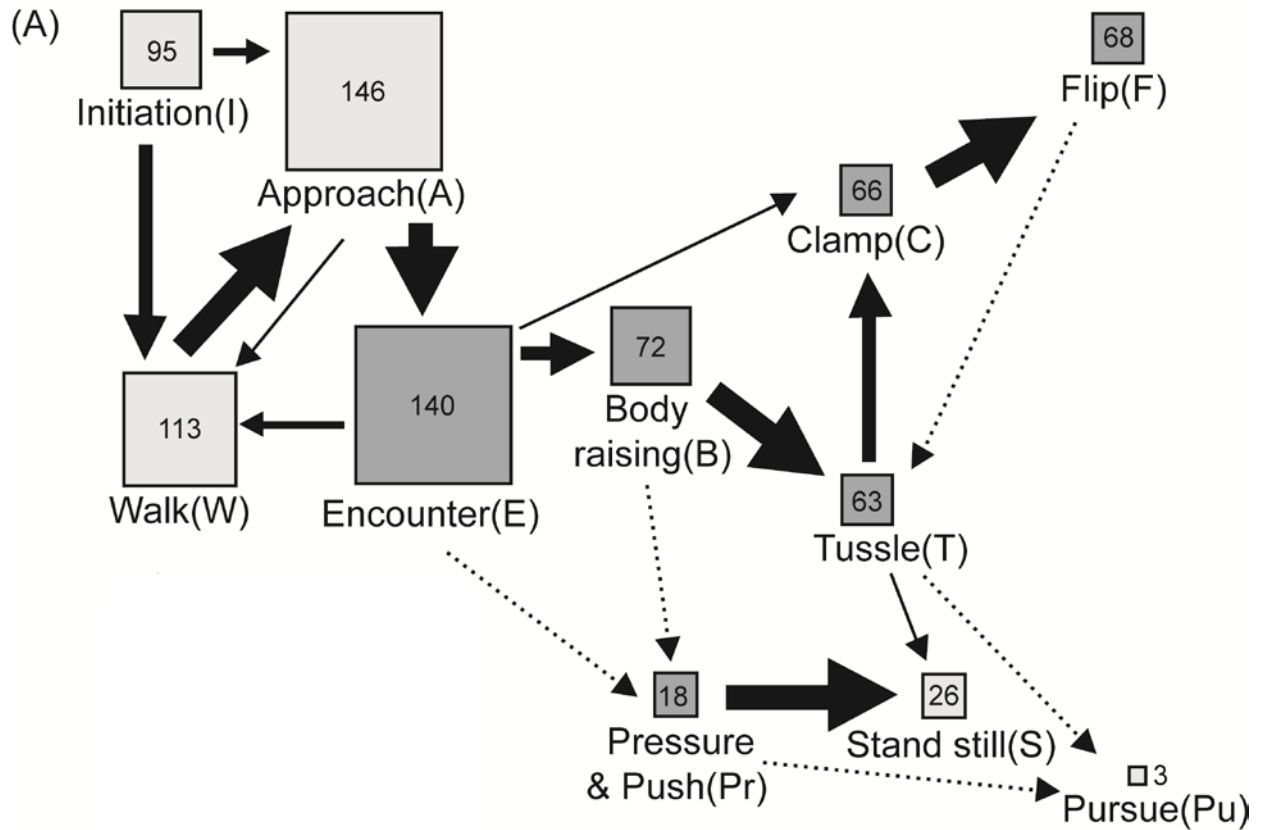


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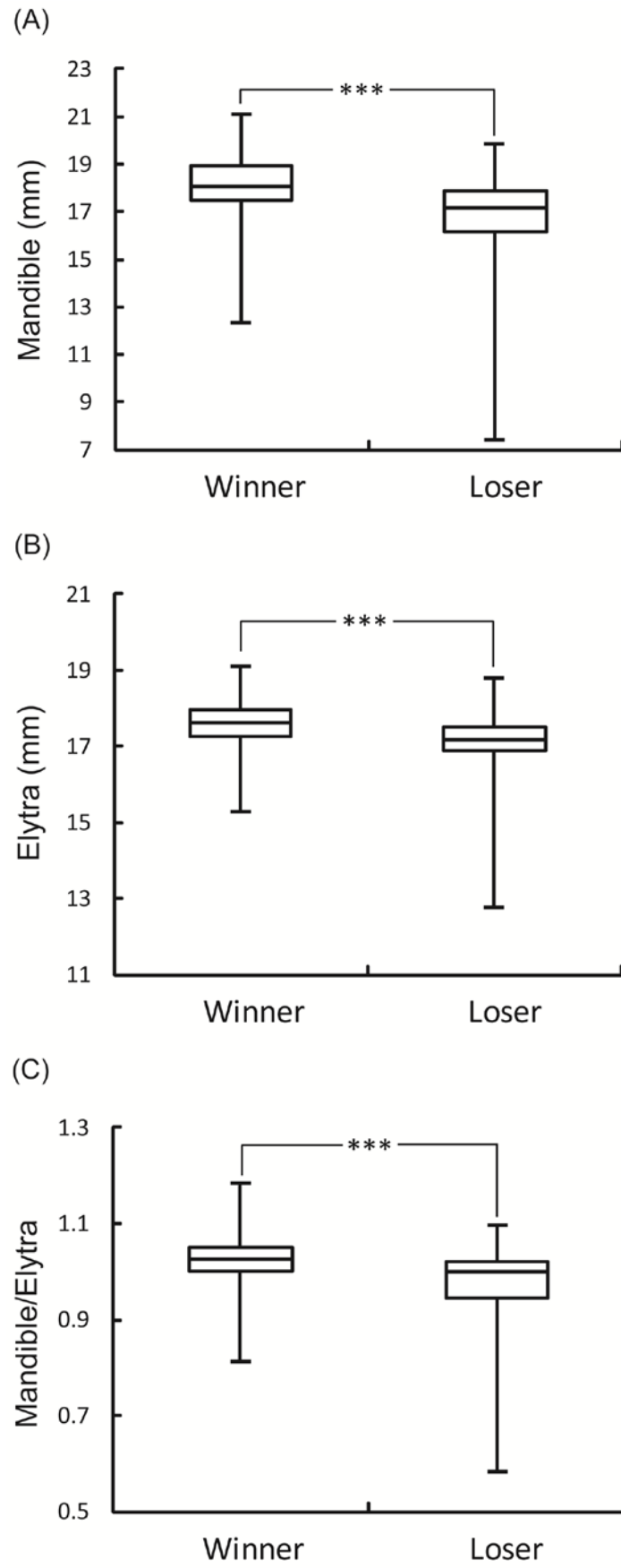


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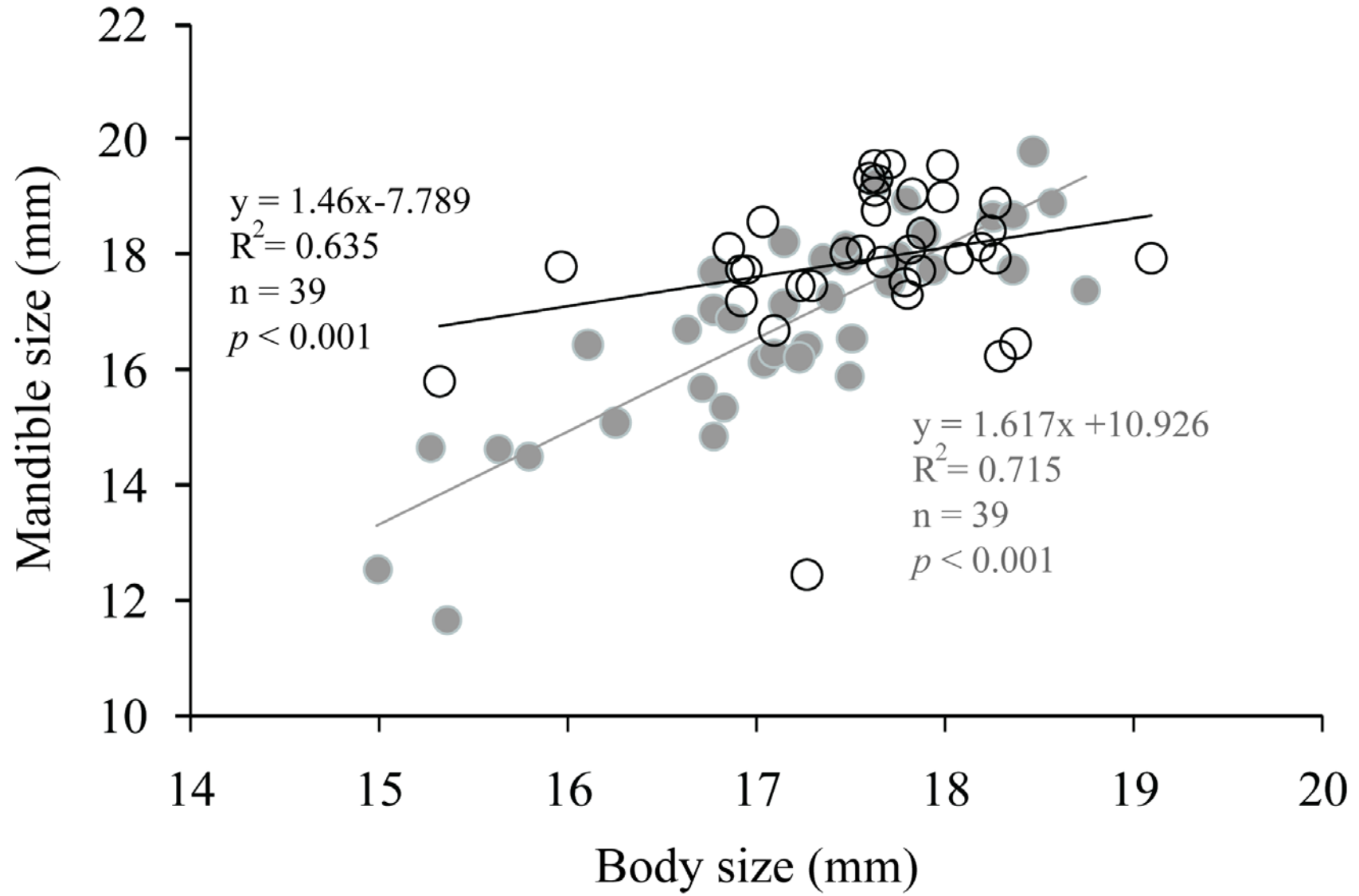
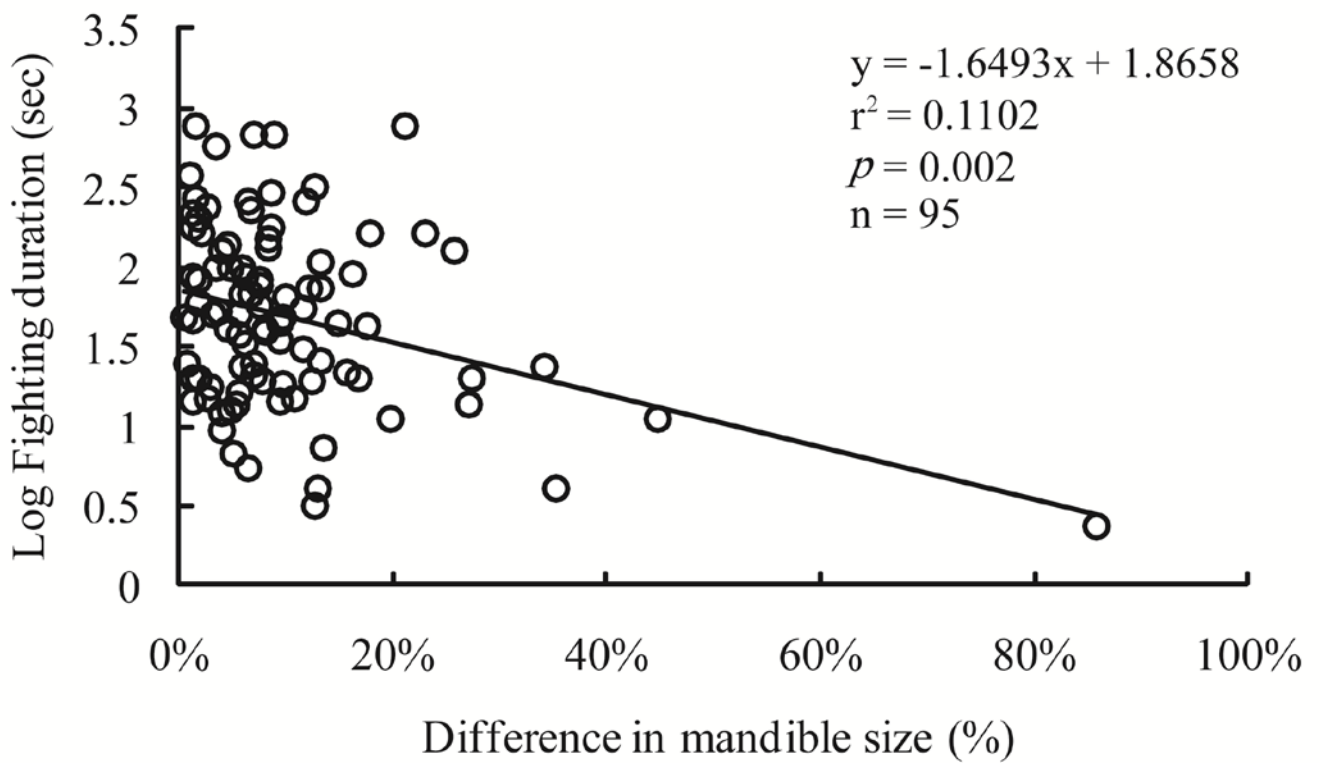


Figure 8

(A)



(B)

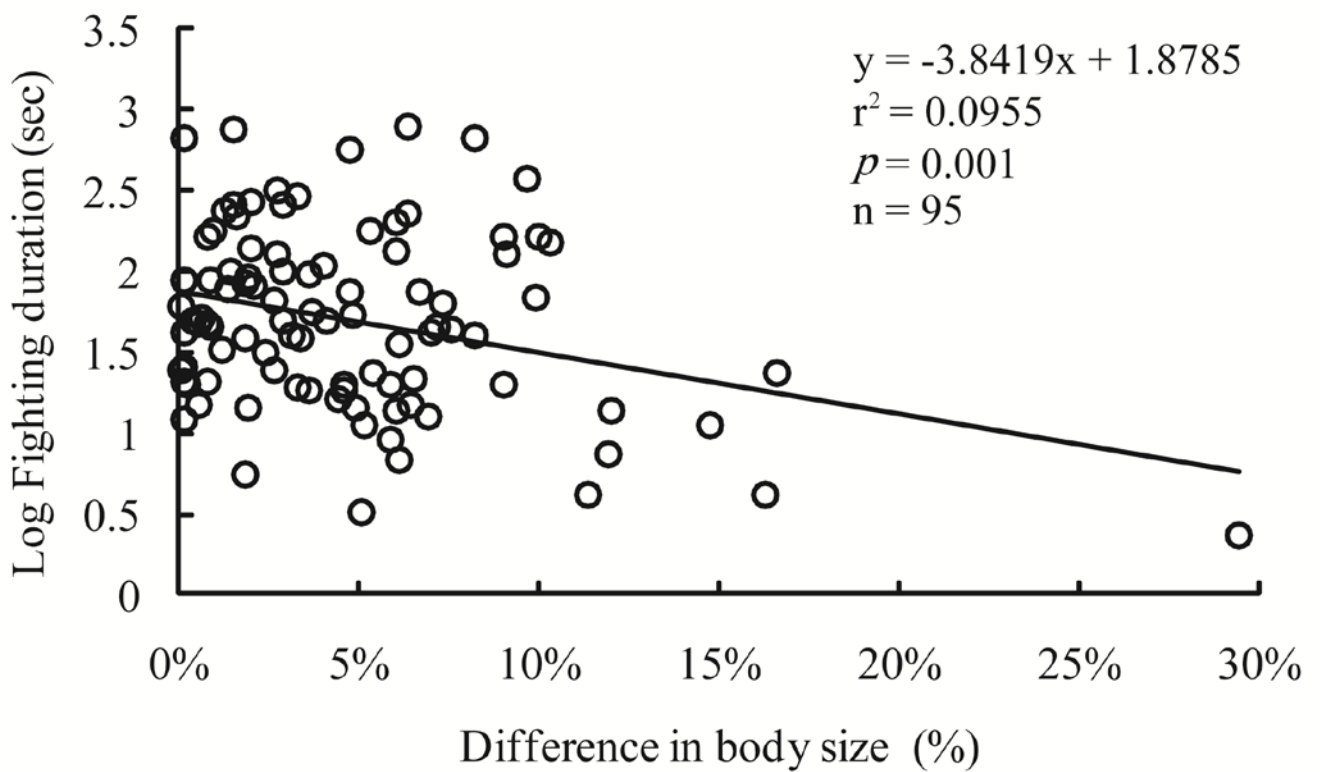


Figure 9

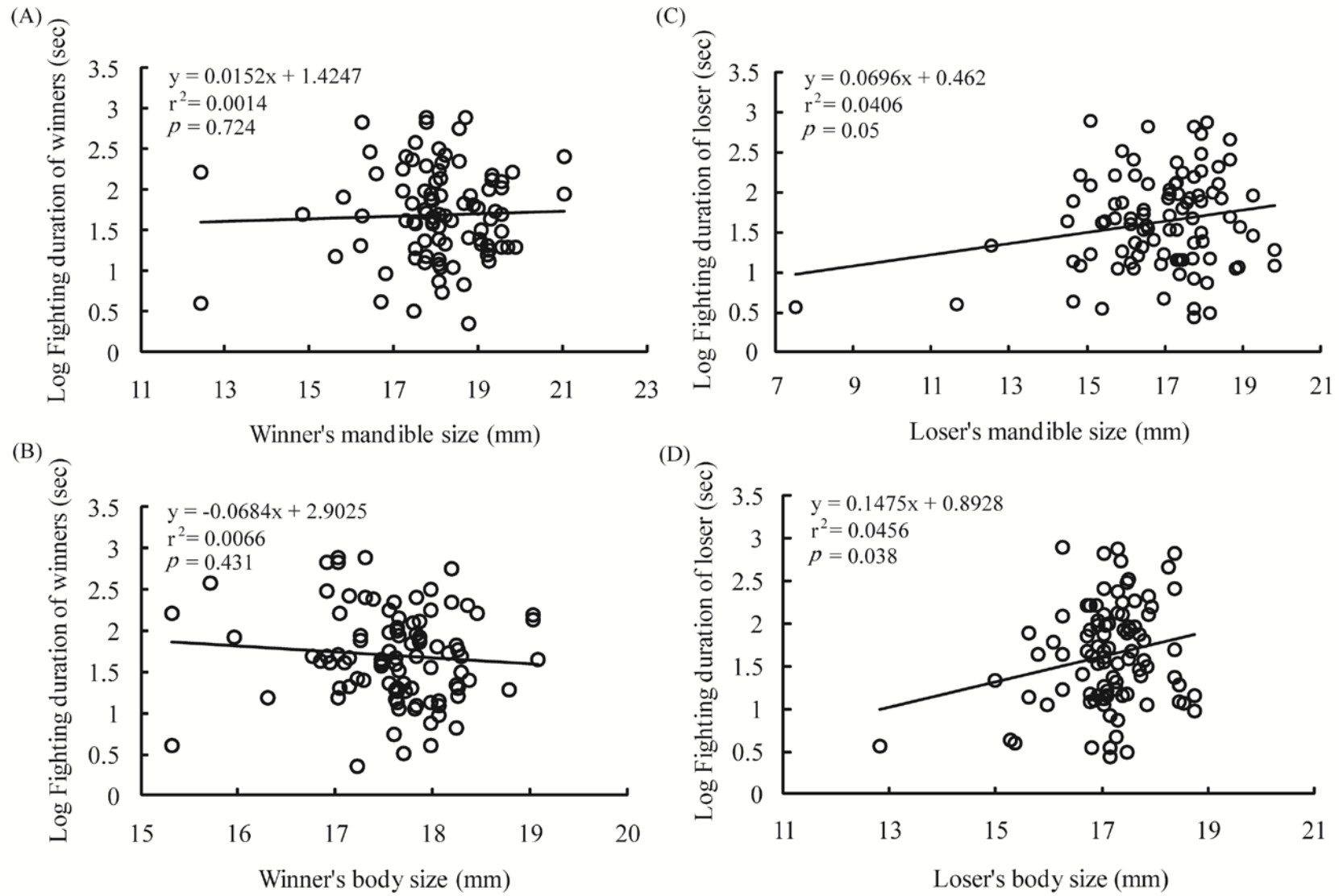


Figure 10

