2019

SHAPING THE TOOLS OF BATTLE : SEXUAL SELECTION, CONTEST DYNAMICS, AND WEAPON DIVERGENCE IN THE ASIAN RHINOCEROS BEETLE TRYPOXYLUS DICHOTOMUS

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By

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B.A., Hendrix College, Conway, AR, 2015

Thesis

presented in partial fulfillment of the requirements
for the degree of
M.S.
in Organismal Biology, Ecology, and Evolution

The University of Montana
Missoula, MT
May 2019
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Chapter 1

Contemporary Sexual Selection Matches Weapon Size Divergence In a Rhinoceros Beetle

Jillian F. del Sol

Abstract

Exaggerated weapons of sexual selection often diverge more rapidly and dramatically than other body parts, suggesting that relevant agents of selection may be discernible in contemporary populations. I examined the ecology, reproductive behavior, and strength of sexual selection on horn length in five recently diverged beetle populations that differ in relative horn size. I show that mating system ecology differs between these locations and corresponds with the local strength of contemporary selection on horn length. Comparisons of ecological conditions and selection strength across populations offer a critical first step towards meaningfully linking mating system dynamics, selection patterns, and diversity in sexually selected traits.

Key Words

Sexual Selection, Male Competition, Mating System, Weapon Evolution

Introduction

  Sexually selected weapons used in male-male competition include some of the most ornate and diverse traits in the animal kingdom (Andersson 1994; Emlen 2008). Despite their shared purpose as tools of mating competition, weapons differ dramatically in shape, size, position/placement on the body, and elaboration between closely related taxa (Rosenberg 2001; Caro et al. 2003; Emlen et al. 2005; Bro-Jorgensen 2007; Schutze et al. 2007; Emlen 2008; Painting et al. 2014; McCullough et al. 2015)Their extreme size and rapid diversification imply a history of strong and likely divergent selection, but the elaboration and diversification of these structures are not well understood (e.g., West-Eberhard 1983; Kingsolver et al. 2001; McCullough et al. 2016). Surprisingly few studies have quantified selection acting on weapons in the wild, and results to date vary (Coltmann et al. 2002; Painting et al. 2015; Zeh et al., 1994; Kelly 2004; Hongo 2007; Kruuk et al. 2002; Wellborn 2000; Reviewed in O'Brien 2017), with many studies failing to detect evidence of any selection on weapons in contemporary populations (Poissant et al. 2008; Kim et al. 2011; Painting and Holwell 2014). This suggests that sexual selection on weapons is episodic, or that costs of these structures sometimes offset benefits, resulting in balancing net selection on weapon form (O'Brien et al. 2017). Differences in selective regimes on weapons may serve as a mechanism generating their diversity across clades. Identifying contemporary selective regimes and the factors that shape them could offer insight into the drivers of early stages of weapon divergence.

  Conspecific populations may differ in the intensity or nature of selection acting on male weapons, providing opportunities to test critical predictions of mating system theory, and to explore ecological factors responsible for driving the initial stages of weapon divergence (West-Eberhard 1983; Bond[uriansky and Day 2003; Kodric-Brown and Brown; Endler 1983; Wellborn 2000; Miller and Svensson 2014). Here, I have capitalized on among-population variation in the relative size of a male weapon, the forked head horn of the Japanese rhinoceros beetle, to test whether patterns of contemporary selection in the wild are consistent with local differences in relative weapon size. Specifically, I explore the ecology, reproductive behavior, and strength of sexual selection acting on horn length in five recently diverged beetle populations, three with relatively short and two with much longer horn sizes. I show that mating system ecology differs between these locations and corresponds with the local strength of contemporary selection on horn length.

The Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Dynastinae), is a univoltine scarab found in broadleaf forests across the Asian continent (Enrodi 1985). This species has been studied most extensively on Honshu Island, Japan, where adults emerge from the soil during summer months and fly to wounds on the sides of mature oak, ash, and maple trees (e.g., *Quercus mongolica*, *Q. acutissima*, *Q. serrata*, *Fraxinus griffithii, Acer plantanoides*; Hongo 2007) and feed on oozing sap (Siva-Jothy 1987; Setsuda *et al.,* 1999; Hongo 2003, 2007). Males battle with rival males for residency at these feeding territories (Obata & Hidaka 1983; Siva-Jothy 1987; Hongo 2003, 2007). Feeding sites are scarce, and therefore comprise limiting, localized and economically defensible resources, which are the predicted drivers of resource-defense systems like that of *T. dichotomus* (Emlen 2008). Fights between males are frequent (Fig. 2), and males with relatively larger body sizes and longer horn lengths are most likely to win (Siva-Jothy 1987; Hongo 2003, 2007, 2010; Karino *et al.*, 2005, Fig. 4). Females mate with males at these feeding territories before leaving to lay eggs in decomposing litter up to a kilometer or more away (McCullough *et al.*, 2012; McCullough 2013).

Like most 'exaggerated' sexually selected weapons (Kodric-Brown & Brown 1984; Zeh & Zeh 1988; Iwasa & Pomiankowski 1999; Biernaskie et al., 2014), *Trypoxylus* horn size is strongly condition dependent (Karino et al. 2004, Emlen et al., 2012; Johns et al., 2014; Kojima 2015). Males modulate weapon growth in response to larval nutrition in a manner consistent with a developmental norm of reaction (Karino et al. 2004; Johns et al. 2014). Static scaling relationships between horn length and body size thus approximate the average underlying reaction norms between horn length and nutrition for a population (Emlen & Nijhout 2000; Shingleton et al., 2007; Dreyer et al. 2016; Mirth et al. 2016; O'Brien et al. 2017).

The populations included in this study differ in their respective norms of reaction such that males in three locations (Puli and Chia-yi, Taiwan, and Yakushima Island, Japan) have horn lengths that are relatively shorter than males in the remaining two locations (Kameoka and Kyoto, Honshu Island, Japan; Figure 1). I use existing data from one of the long-horned populations (Kyoto; Hongo 2007) and season-long observations of male mating success, behavior, and ecology at the remaining four populations, to test whether the degree of resource limitation and resulting intensity of sexual selection was stronger in long-horned populations than in the short-horned populations.

Methods and Materials

Study locations.

Ecological and behavioral observations were conducted at four populations, and these data were analyzed alongside prior data collected in Kyoto, Honshu, Japan between 2003 and 2006 (Hongo 2007). The active breeding seasons at all locations lasts approximately 1.5 months. However, since Taiwan is located at least 7 degrees latitude closer to the equator than the other locations, the breeding seasons of the two Taiwan sites started almost two months earlier than the other locations. This staggered onset of breeding permitted me to observe mating behavior at two different locations each year, one in the south (Taiwan) and the other farther north (Yakushima; Kyoto).

In the summer of 2016, observations were conducted first on the forested campus of National Chi-Nan University, located in Puli, Taiwan (June-July), and then, in the second half of the summer (late July- August), on the island of Yakushima, Japan. In 2017, observations were conducted first on the forested campus of Chia-yi University in Chia-yi Taiwan; then later in the summer, beetles were observed in a deciduous forest in Kameoka, Japan.

Observation, morphological measurements and scaling.

Observations began around 19:00 each night (prior to dusk and beetle emergence) in order to allow researchers to capture unmarked males upon their arrival at active trees prior to their involvement in relevant behaviors. Males were gently captured with a net or gloved hands, and were quickly measured with dial calipers (Anytime Tools, CA, USA) and marked with a unique number on both elytra with a non-toxic paint pen (Blick Art Supply, IL, USA). Horn length was recorded with one end of the calipers at the clypeal projection near the mouthparts, and the other end extended to the tip of the inner right horn projection to capture the full functional length of the structure (Hongo 2007). Two measures of body size were recorded: elytral length (a straight line from the top of the scutellum to the bottom corner of the right elytron) and the maximum width of the pronotum (Supp. Fig. 1). Observations were conducted using headlamps with built

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in red filters (RioRand, USA). Light beams were focused adjacent to beetles to minimize disturbance, and were turned to the lowest setting (200lm) whenever possible.

Population Density and Resource Distribution.

As with many beetles (e.g., Gries & Gries 1994; Rochat et al., 2004; Wertheim 2005), adult *Trypoxylus dichotomus* respond to aggregation pheromones and collect in local areas of high activity. Thus, even when putatively suitable host trees exist over wide areas, beetles tend to converge on a few focal host trees, resulting in locally dense populations that are widely separated from other such populations. Often, these exact localities are used by beetles in multiple years, resulting in stable hot-spots of beetle activity (e.g., Hongo 2003, 2007, 2012).

At each of our study locations we identified the local hotspot of activity and quantified ecological variables pertinent to the likely intensity of mate competition. These included the species of host tree, counts of the number of host trees with active territories, and nightly estimates of the number of available territories. Additionally, I conducted hourly counts of the numbers of males and females at territories, providing both nightly and cumulative estimates of overall beetle densities and local sex ratios. In addition to these population-wide measures, I and an undergraduate assistant conducted hour-long focal animal observations of males (Altmann 1974) to measure the frequency and type of interactions with competitors and potential mates.

Because only reproductively active adults fly to feeding locations (Siva-Jothy 1984), I estimated the operational sex ratio as the average ratio of adult males to adult females at the breeding aggregations each night. I estimated the value of territories at each location as the average number of female visits per hour observed during our focal male observations. The cost of guarding a territory was measured as the average number of approaches and/or challenges by rival males per hour. Finally, we estimated the opportunity for selection by calculating the variance in male mating success as described by Shuster and Wade (2003).

Focal animal observations were supplemented by regular population-wide censuses of matings, as successful copulations were infrequent enough that they were unlikely to occur during the focal male observations, and because we wanted to identify, as best as possible, every successful mating occurring in the local breeding assemblage during the season as they occurred (see "*Selection on horn length in the wild*", below). Laboratory studies suggest that successful

sperm transfer requires a minimum of a half hour *in copula* (range 30 -150 minutes; Karino & Niiyama 2006) and, in the one field location examined prior to this study, copulation durations in the field typically lasted 30 min or longer (Hongo 2007). Thus, I surveyed all active trees every half hour throughout each night, locating every courting and copulating pair and recording the identity of mating males. Because successful copulation is associated with an easily recognizable posture distinct from courtship (Fig. 2), it is possible to discern at a distance any pairs of beetles *in copula* as well as the elytral ID number of marked males. In this way, our population surveys are likely to have captured the majority of successful copulations in the population during the entire breeding season.

Function of male horns in combat.

We used our focal male observations to measure the stages of escalation and outcome of male - male interactions at territories (del Sol et al in prep.). Additional sampling of fights was conducted intermittently throughout the season. For the present study, I confirmed that males use their horns in agonistic interactions with rival males at all study locations. To quantify the effect of body and horn size on fight outcome, I modeled the likelihood that the focal male wins using the predictor variables of the differences in horn or body size in a logistic general linear model for each site (Hardy and Briffa 2012; Painting et al. 2015).

Statistical Analyses- Comparing relative horn length across populations.

All statistical analyses were conducted in opensource RStudio (R v. 3.5.3 ; RStudio v. 1.1.463; R Foundation for Statistical Computing, 2016). To compare the relative weapon sizes of males in different populations in a biologically relevant manner, I regressed horn length on pronotum width, and compared linear, logistic, and Gompertz line fits using AIC for each population size (Nijhout and German 2012; Fig. 1). Population model pairs (both for log-transformed linear and logistic models) were compared using the Likelihood Ratio test to confirm the "long" and "short" –horned designation for each population. Residuals for selection models were calculated using the model with the lowest average AIC across populations.

Statistical Analyses- Selection on male horn length in the wild.

As described in O'Brien et al (2017), selection on an exaggerated male weapon can be measured as a metric trait experiencing direct selection acting on absolute weapon size, or considering the highly phenotypically plastic nature of this trait, measuring instead bivariate selection on the relationship between weapon and body size (a 'reaction norm' perspective *sensu* Emlen and Nijhout 2000; Shingleton et al. 2007; Dreyer et al. 2016; Mirth et al. 2016; O'Brien et al. 2017). For each study population, I tested five hypotheses for selection on the head horn in male *Trypoxylus*: (I) directional selection for larger absolute weapon size, (II) stabilizing selection on absolute weapon size, (III) proportional selection on reaction norms (increased reaction norm intercept), (IV) correlational selection on reaction norms (increased reaction norm slope), and (V) stabilizing selection on a the population reaction norm. Using field measures of mating success from our population-wide censuses, we tested for each form of selection in the four study locations for which we obtained mating data.

Morphological measures were mean standardized before analysis (Lande and Arnold 1984). Logistic regressions were used to assess allometric relationships between horn length and body size (pronotum width) in each population given the approximately equal error in measures of trait and body size ("smatr" package in R, Warton, D. [2005]). Residual values from logistic models were collected as a proxy of relative weapon size, since the logistic models described the horn-body size relationship most closely.

Ordinary least squares (OLS) regression was used to determine support for Hypotheses I-V in accordance with the methods described by Lande and Arnold (1983) and Arnold and Wade (1984). Models only incorporating linear terms were used to assess directional selection. Models incorporating both linear and quadratic terms of the explanatory variable were used to assess patterns of stabilizing selection. In each analysis, "mating success" referred to relative mating success as a continuous response variable. Relative mating success was calculated for each male as the number of observed successful matings across the entire breeding season divided by the average number of matings per male in the population across the entire breeding season. Akaike information criterion (AIC) was used to compare models.

Hypothesis I, directional selection for absolute weapon size, was assessed by regressing mating success on absolute weapon size. Hypothesis II, stabilizing selection on absolute weapon

size, was assessed by regressing mating success on absolute weapon size using both linear and quadratic representations of weapon size. Hypothesis III and IV were assessed by regressing relative mating success on weapon size, body size, and the interaction between weapon and body size. Hypothesis III, correlational selection on reaction norms, was assessed using regression coefficients representing the interaction between weapon and body size. Hypothesis IV, proportional selection on reaction norms, was assessed using the regression coefficients representing weapon size after controlling for body size. Hypothesis V, stabilizing selection on reaction norms, was assessed by regressing relative mating success on relative weapon size using both linear and quadratic coefficients as representations of relative weapon size.

To further distinguish between these hypotheses, I assessed relative mating success as a function of absolute residual weapon size. This allowed me to assess the presence of stabilizing selection without incorporating quadratic coefficients into our analyses. In addition, I analyzed differences in variance in relative weapon size between mated and non-mated males in the population (treated as a binary response variable where males either successfully or unsuccessfully inseminated at least one female throughout the breeding season) using Levene's test (using residual values from MA regression). As stabilizing selection is predicted to reduce variation within a population, we believe this comparison valuable in assessing such patterns in wild populations (e.g., O'Brien et al. 2017).

Finally, selection differentials and gradients were calculated for both absolute and relative weapon size to compare the strength of selection associated with Hypotheses I and III across our study populations. Selection differentials were calculated as the difference in mean absolute weapon size of mated and non-mated animals. Selection gradients were calculated as the partial regression coefficients from OLS regression of relative mating success on absolute weapon size (Lande and Arnold 1983).

Results

Populations differ in relative horn length

Based on the visual 95% confidence as well as differences in log-likelihood ratio test, Kameoka and Honshu populations are indeed longer horned than Puli, Chia-yi, and Yakushima, which overlap as 'short-horned' (Fig. 1).

Mating system characteristics and ecology vary between locations

Overall, the mating behavior of beetles was similar across locations, in that adult males and females emerged from litter at dusk and flew to host trees with active territories to feed. Males encountered rival males on the sides of trees and battled over ownership of territories, and females encountered males on the trunks of trees and were courted and mated while feeding. After feeding, females left the breeding aggregations to seek out nearby locations with rotting vegetation where they lay their eggs (e.g., Hongo 2003, 2007; McCullough radio telemetry paper). In contrast, males tended to remain at the breeding aggregations throughout the night, and returned for successive nights until they were eaten or died of other causes. Consequently, males tended to remain at the breeding aggregations for longer than females, contributing to operational sex ratios that were male biased (Table 1).

Although the general features of the mating system appeared similar at all study locations, some details differed. The species of host tree differed, with beetles in the two long-horned populations (Kyoto, Kameoka) using Oak (*Quercus* spp.), beetles in two short horned populations (Taiwan- Puli, Chia-Yi) using ash trees (*Fraxinus griffithii),* while in Yakushima, beetles fed primarily on sap flows carved into bay trees (*Machilus thunbergii*) by unidentified heterospecific larvae. In addition, the overall densities of beetles differed dramatically, with the Puli, Taiwan population having the most animals (up to 300 animals at the breeding aggregation per night and 900+ individuals throughout the season) and Chia-Yi and Yakushima having the fewest (approximately 25 and 8 beetles at the aggregation per night, respectively) (Table 1).

Beetles in both long-horned populations feed on oak sap flows created sporadically by burrowing moth larvae during summer months (Hongo 2005, 2007). Ostensibly due to small moth populations, these sap flows are rare, often concentrated to a few trees in a given area, and can stop flowing at any time if the larvae stop feeding. In the present study, a single tree hosted the entire breeding aggregation in Kameoka with an average of 3 territories active in a night, and in Hongo (2007), there were 7 active trees in an entire experimental forest. In contrast, beetles in short-horned populations feed on *Fraxinus* (Taiwan populations) and *Machilus* (Yakushima, JP), thin-barked trees that allow both male and female *T. dichotomus* to carve sap flows at will using clypeal projections (Hongo 2005). Because beetles could carve their own territories, feeding sites were much more abundant- often with a dozen or more per tree and many trees with available

feeding sites. Abundance of territories and the number of flows per male competitor were much higher in all three short-horned populations than in the long-horned, likely a result of nightly bark- carving by both males and females (Table 1).

In order to combine the effects of beetle density and feeding territory availability into biologically meaningful metrics of the social environment relevant to selection on horns, I used over 350 hours of focal animal observations of territorial males (these data were not collected in Hongo's original study, so we do not include Kyoto in these comparisons). In this way I could estimate the average number of times a territory-holding guarding male was likely to be confronted by a rival male (and estimate of the relative cost of guarding a territory in each location), as well as the number of times a territory was likely to be visited by a female (an estimate of the relative benefits of guarding a territory in each location).

Males were likely to face multiple challenges by rivals each night at all of the populations (12.2 +/- 3.3 challenges per night at Kameoka; 27.8 +/- 4.25 at Puli; 2.9 +/- 1.1 at Chia-Yi; and 3.4 +/- 0.9 at Yakushima; Table 1), despite the fact that several of these populations had huge numbers of available territories (e.g., 612.5 +/- 51.9 territories per night at Puli, and 168 +/- 3.6 territories per night at Yakushima), and one of these (Yakushima) also had very low overall numbers of beetles (approximately 11 adult beetles per night). This suggests that beetles are congregating at sites occupied by other beetles, and is consistent with the presence of an aggregation pheromone in this and other rhinoceros species (e.g., Gries and Gries 1994; Hallett et al., 1995).

Puli, Taiwan, had extraordinarily high densities of beetles and this resulted in the highest per-night number of challenges to guarding males. Interestingly, Kameoka, a long-horned population, also had high numbers of challenges per night, despite having the lowest overall number of adult beetles in the local aggregation (approximately 8 adults per night), presumably reflecting the severe limitation of available territories (table 1).

Males guarding territories at the one measured long-horned population (Kameoka) were far more likely to be visited by female beetles than were males guarding territories at the three short-horned locations (12.6 +/- 3.3 female visits per night, compared with 4.6 +/- 0.6, 3.7 +/- 0.7, and 0.7 +/- 0.3 female visits per night at Puli, Chia-Yi, and Yakushima, respectively), suggesting that the costs of guarding a territory are most likely to be offset by reproductive

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benefits at locations where available feeding territories are most severely limiting (Table 1; Supp. Fig. 2).

Contest dynamics are similar across populations

At all five study locations males fought rival males over possession of sap sites. Males turned to face approaching rivals upon contact and used horns to jab and lunge in response. In escalated fights, males attempted to use their forked head horn to pry opponents off of the tree. The logistic regressions showed that larger and longer horned males are more likely to win fights in each population (Fig. 4). Since horn and body size are tightly correlated (Fig. 1), a GLMM with horn size and site as fixed variables and individual beetles as random effects is sufficient (AIC differences < 1) to show the significance of horn size in predicting contest outcome ($p=0.01$) and shows that outlier beetles or individual fights do not impact the model. This model also shows one site effect of Kameoka ($p = 0.0082$), in accordance with this population's tendency for a lower difference between winners and losers (see Chapter 2, supplementary figure 2).

Populations differed in the intensity of net selection acting on male horn length

Using lifetime mating success as the measure of male fitness (Hongo 2005, 2007; Harada & Fujiyama 2017), we found remarkably strong selection (as defined by Kingsolver et al. 2001) for longer horns in both long horned populations (Kyoto: $\beta=0.43 \pm 0.09$, $p < 0.001$; Kameoka: $\beta =0.65 \pm 0.28$, $p< 0.05$; Table 3; Fig. 5). In contrast, and in accordance with predictions, one short-horned population experienced weaker directional selection on horn length (Chi-Nan, $\beta =0.27 \pm 0.14$, $p<0.05$), and I did not detect significant directional selection on horn length in the second short-horned population (Chia-yi, $\beta =-.33 \pm 0.08$, $p > 0.1$). Despite conducting night-long observations throughout the season, I did not observe any mating events at one short-horned population (possibly due to low population sizes; Table 1), so were not able to calculate selection gradients. We did not detect significance in any gamma term or for directional selection on residual weapon size, allowing us to reject hypotheses of selection acting on the reaction norm in these populations.

Discussion

I observed fighting and mating behavior at four locations across the range of the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (National Chi-Nan University, Puli, Taiwan [June-July 2016]; Yakushima Island, Japan [late July- August 2016]; Chia-yi, Taiwan [June-July 2017]; and Kameoka, Honshu, Japan [late-July-August 2017]), and combined my findings with the results of two years' observations at a fifth location, Kyoto, Honshu, Japan [July-August 2003, 2006; Hongo 2007). Essential features of the mating system, and general behavior of beetles, were consistent across all five locations. Invariably, beetles aggregated at a cluster of suitable host trees, flying to the sides of the trees at dusk and fighting, feeding, and mating at wounds on the sides of these trees. At all locations males battled with rival males over access to these feeding territories regardless of territory availability (Table 1), and males with the largest body sizes and longest horn lengths were the most likely to win (Figure 3). Females visited trees to feed at the sap sites before leaving to oviposit in the surrounding litter. At all locations, females encountered males at the feeding sites, and all matings occurred at or near these territories.

Classical mating system theory predicts that the intensity of sexual selection will depend on the extent to which breeding resources—high quality territories or females— are defensible and valuable enough to be worth sequestering (Brown and Orians 1970; Emlen and Oring 1977; Shuster and Wade 2003). We observed male-biased operational sex ratios at all of our study locations (Table 1), presumably reflecting the fact that males tend to remain at the territories all night, for as many successive nights as they are able, whereas females visit feeding territories for only up to three days to feed and mate before flying away to oviposit in the surrounding litter (Hongo 2007; McCullough 2013). Observed operational sex ratios ranged from 1.5 to 5.0, and, interestingly, were lowest at one of the long-horned populations, suggesting that average nightly OSR is not a good predictor of the local intensity of sexual selection acting on male horns.

Beetle density and the number of available territories each varied extensively across our sites, and both appear to contribute to the relative cost males pay for successfully guarding a territory at each location. Specifically, populations with large numbers of rival males (Puli) and/or severe limitation of available territories (Kameoka) had the highest number of challenges per night faced by territory-guarding males. We suggest this provides a relevant metric for contrasting the relative "price" males must pay for holding a feeding territory. of territory defense would be reflected in our season-long measures of male mating success and net selection

on horns. Despite low numbers of beetles (and correspondingly small final sample sizes) at several locations, our results confirm these predictions.

Although the general features of the mating system were similar across sites, many of the details differed (Table 1, Figure 4), and these differences are likely to be relevant to the intensity of sexual selection acting on horns at each location. Most notably, the species of host tree differed from location to location (Oak [*Quercus spp*.] at Kameoka and Kyoto, and Ash [*Fraxinus griffithii*] at Puli and Chia-yi, and Bay [*Machilus thunbergii*] at Yakushima). This is key to changing resource ecology because of the specific feeding behavior of *Trypoxylus dichotomus*. Their ability to carve only certain host trees means that, at the two Honshu locations (Kameoka and Kyoto), beetles must rely on wounds created by the boring larvae of a moth (Hongo 2003, 2007), unable to carve into thick oak bark. Thus, animals are forced to compete for a small number of already-present wounds on the sides of the trees (~4 per site in Kameoka and ~3 per site in Kyoto). As a result, territories were severely limiting at these locations and the intensity of competition over these sap sites was accordingly high.

At the other three locations, bark on host trees was much thinner, and the only oak trees are introduced artificially. As originally proposed by Hongo (2007), thinner bark allows beetles to carve their own territories instead of expending energy by fighting for them. Consequently, although the basic behaviors of the beetles were similar -- males still fought rival males and females still fed at sap sites before flying off to lay eggs -- the number of available territories was much higher (Table 1). With territories less limiting, the relative value of territory ownership decreased. Specifically, the probability that a male was challenged by a rival was almost as high at Puli and Chia-Yi, two short-horned populations, as it was at the long horned populations on Honshu (number of challenges on average at Puli = 27.8+/-4.25; at Kameoka, males faced half as many challenges, 12.2+/-3.3), but the probability of a territorial male being visited by a female was lower (Table 1, Figure 3). While males with longer horns experience *competitive* benefits in each population, territorial males enjoyed the greatest reproductive benefits of territory holding in the long-horned population for which we obtained ecological data. The number of females that a male was likely to encounter was significantly higher in the long-horned population than in any of the short-horned populations (Fig. 3), translating both into population-based differences in the opportunity for selection (Fig. 1) and in the comparative strength of selection acting on horn length (Table 2; Table 3).

Peculiarities of this system means that measuring male fitness via their mating success is unusually reliable (Hongo 2005, 2007; Harada & Fujiyama 2017), given the tendency for females to mate singly despite being courted by many males. As predicted, we found remarkably strong selection (as defined by Kingsolver et al. 2001) for longer horns in both long horned populations (Kyoto: $\beta=0.43 \pm 0.09$, $p < 0.001$; Kameoka: $\beta = 0.65 \pm 0.28$, $p < 0.05$; Fig. 3). This is consistent with the selective benefits afforded by high quality territories in these populations. In contrast, and in accordance with predictions, one short-horned population experienced weaker directional selection on horn length (Chi-Nan, $\beta = 0.27 \pm 0.14$, $p < 0.05$), and we did not detect directional selection on horn length in the second short-horned population (Chia-yi, $\beta = -.33 \pm 0.08$, $p > 0.1$). In Yakushima, territory quality was so low that we were not able to observe any mating events. In this site, hundreds of active territories were counted over the season, while we routinely counted fewer than 10 beetles nightly across a 7 kilometer "hotspot".

Measures of direct selection on absolute weapon size are the most intuitive way to quantify the strength of selection acting on an exaggerated sexually-selected structure. However, given that most of these structures are highly nutrition- and condition-sensitive in their expression (Kodric-Brown & Brown 1984; Zeh & Zeh 1988; Iwasa & Pomiankowski 1999; Biernaskie et al., 2014), a more appropriate approach, particularly for insects, where body size is often overwhelmingly influenced by larval access to nutrition, is to consider static scaling relationships- a population level measure- as approximations for underlying developmental reaction norms (Emlen & Nijhout 2000; Shingleton et al., 2007; Dreyer et al. 2016; Mirth et al;., 2016; O'Brien et al. 2017).   Sexual selection would then act on either the slope or intercept of these scaling relationships by favoring males with relatively longer horns for their body size.

Both horn length and body size in *T. dichotomus* are nutrition- and condition-sensitive (Karino et al. 2004, Emlen et al., 2012; Johns et al., 2014; Kojima 2015). Since T. dichotomus subpopulations, like other weaponed species complexes, differ in the size of weapons relative to the body (Fig. 1), I predicted that the processes that act to differentiate weapon phenotype are likely acting on relative horn length, in accordance with theory suggesting that selection shaping such patterns should act on relative weapon size (Bonduriansky and Day 2003; Dreyer et al. 2015). I also tested for stabilizing selection on local patterns of horn length relative to body size to assess the alternative hypothesis that rather than a lack of directional selection, smaller weapons are maintained by differing patterns of stabilizing selection. However, I found no

evidence that non-linear selection is acting on the horn-body size allometry in any of the focal populations.

Using the interaction term in a model regressing relative mating success on male horn length and body size, we tested whether selection might be acting to increase the slope of the horn length - body size scaling relationship (Model 3 in Tables 2 and 3). Similarly, by testing for selection on residual horn length, we tested whether sexual selection might favor increases in the intercept of horn length - body size scaling relationships. Finally, we tested for net stabilizing selection on the reaction norm by including a polynomial (gamma) term in our analyses. Because each of these approaches is likely to be limited by available sample sizes, we also tested for stabilizing selection acting on the horn length-body size reaction norm by taking the absolute value of residual horn lengths and regressing relative male mating success on the extent to which male horn lengths deviated from the population average scaling relationship (O'Brien et al., 2017).

The two years' data collected by Hongo (2003, 2007) at Kyoto, Japan, provided the largest sample sizes, and the clearest picture of selection acting on male weapons. In this population, sexual selection appears to act strongly, favoring the largest males with the longest absolute horn lengths (Tables 2, 3), as well as large males with relatively long horns (Figure 5). Specifically, we find support for models I and III, and treating horn length - body size scaling as a reaction norm, our results suggest selection acts to favor a steeper scaling relationship slope in this location. At Kameoka, the other long-horned population, beetle densities were much lower, and our tests of models of selection on reaction norms were limited by smaller sample size (Table 2). Despite this, we still find evidence for strong net selection on absolute horn length (Table 3, Figure 5). In contrast, selection on horn length was weaker at all three of the short-horned populations, and despite a very large sample size at one location (Puli), we detected no evidence of selection for increased slope or intercept of the scaling relationship between horn length and body size.

These results represent a key piece in understanding the relationship between weapon diversity and the strength of sexual selection in weaponed species[DJ19] . Comparative studies of horned scarabs suggest later mechanisms underlying weapon diversity, like developmental costs (Emlen 2004) and changes in fighting substrate and style (McCullouh 2016). With over 350 total hours of focal male and whole-site observations, my data suggests that selection is not

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being shaped by local differences in horn function, aggressive behavior, or territory substrate, but primarily by the relationship between resource availability and abundance.

The idea that the intensity of mating competition is sufficient to shape sexual selection and resulting phenotypes is pervasive and intuitive. Even in comparative studies that suggest differences in the selective benefits of weapons, researchers consider the outcomes of fights to be a primary driver. Similarly, ecological measures of competition intensity, like competitor density, are commonly invoked as sufficient to drive selection strength (Kokko and Rankin 2006; Kokko 2012). However, this conceptual pattern tends to omit the necessity of a positive relationship between competitive benefits and mating benefits for competition to shape sexual selection. We found no evidence that typical competition drivers- sex ratio, population density- track as expected with the strength of selection on a competitive trait across 4 populations of *T. dichotomus*.

Rather, our results suggest that even when all the conditions for strong selection are met: competition intensity is high, bigger weapons predict competitive success, and mating behavior still reflects a resource-defense system- a negative or nonexistent relationship between competitive ability (territory holding) and mating benefits (the likelihood of female encounters) can result in a collapse of strong selection on weapon size. Changes in the value of a territory, here measured as the frequency of female visitation, appear sufficient to cause this collapse, shaping divergent selection patterns on the same weapon. In this case, this is likely the result of differences in geographically based territory type: the various tree species that beetles feed on in different populations.

By shifting the focus to a species complex at the earliest stages of divergence, my work demonstrates that contemporary differences in the strength of sexual selection are associated with patterns of recent weapon diversity, and suggest that changes in territorial ecology that affect the monopolizability of mates are sufficient to induce these changes. This insight into the role of rapid changes in and response to sexual selection in weapon diversity may come to bear on our understanding of the mechanisms linking the evolution of extreme sexually selected traits and their extreme diversity.

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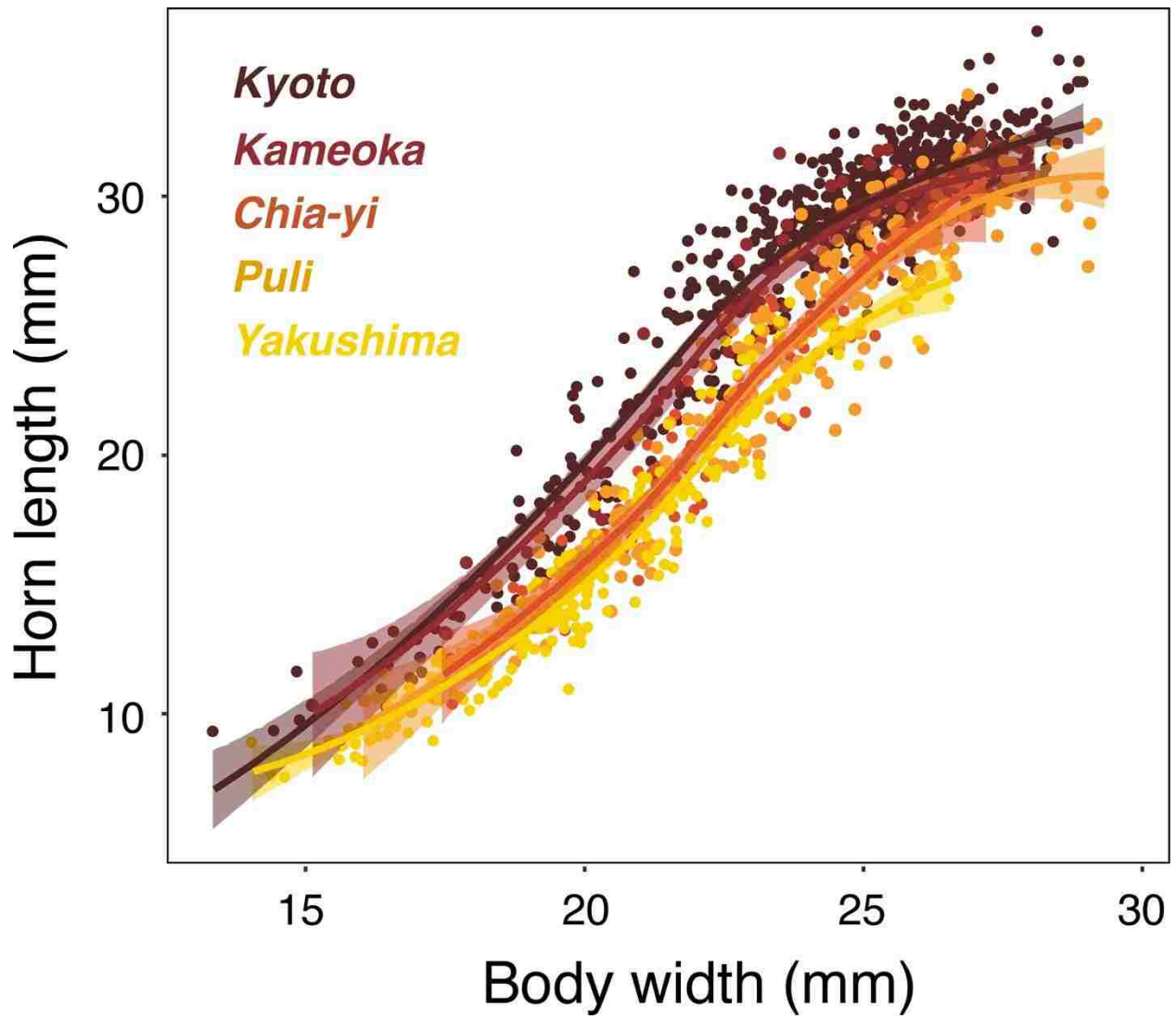
Figure 1: Logistic regressions of body width on horn length for 5 populations of Trypoxylus dichotomus males.

Figure 2: Typical observational conditions and *Trypoxylus dichotomus* behaviors. (A) Two males engage in escalated combat- here, the red "intruder" individual had challenged the territory "owner" while he was courting. The female carves and feeds at the original territory nearby, seemingly unaware of the males. (B) Upon successful insemination, males assume a 'hanging' position. This is distinct from a vertical courtship position, allowing successful mating to be identified from a distance. Both trees shown are *Fraxinus griffithii* in Puli, Taiwan, and illustrate the typical activity of beetles being at or just above eye-level.

Table 1: Mating System Characteristics

| Site Characteristics	Short-horned Populations			Long-horned Populations	
	Puli (Taiwan) 2016	**Chia-Yi (Taiwan)** 2017	**Yakushima Island** 2016	**Kameoka (Honshu)** 2017	**Kyoto (Honshu)** 2003, 2006
Host tree species	Ash *Fraxinus griffithii*	Ash *Fraxinus griffithii*	Bay *Machilus thunbergii*	Oak *Quercus spp.*	Oak *Quercus spp.*
Chew through bark?	Yes	Yes	Yes	No	No
# host trees with active territories	212	26	117	1	7
# active territories per night	612.5 ± 51.9	20.3 ± 3.9	168.2 ± 3.6	4.7 ± 0.17	~3
# males/females at breeding aggregation per night (OSR)	675.5 / 205.3 (3.4 ± 0.26)	15.9 / 9.6 (1.7 ± 0.23)	9.2 / 2.0 (5.0 ± 0.9)	4.3 / 3.5 (1.5 ± 0.14)	N.A.
Territory Characteristics					
# challenges by rival males per night	27.8 ± 4.25	2.9 ± 1.1	3.4 ± 0.9	12.2 ± 3.3	N.A.
# female visits to territory per night	4.6 ± 0.6	3.7 ± 0.7	0.7 ± 0.3	12.6 ± 1.2	N.A.
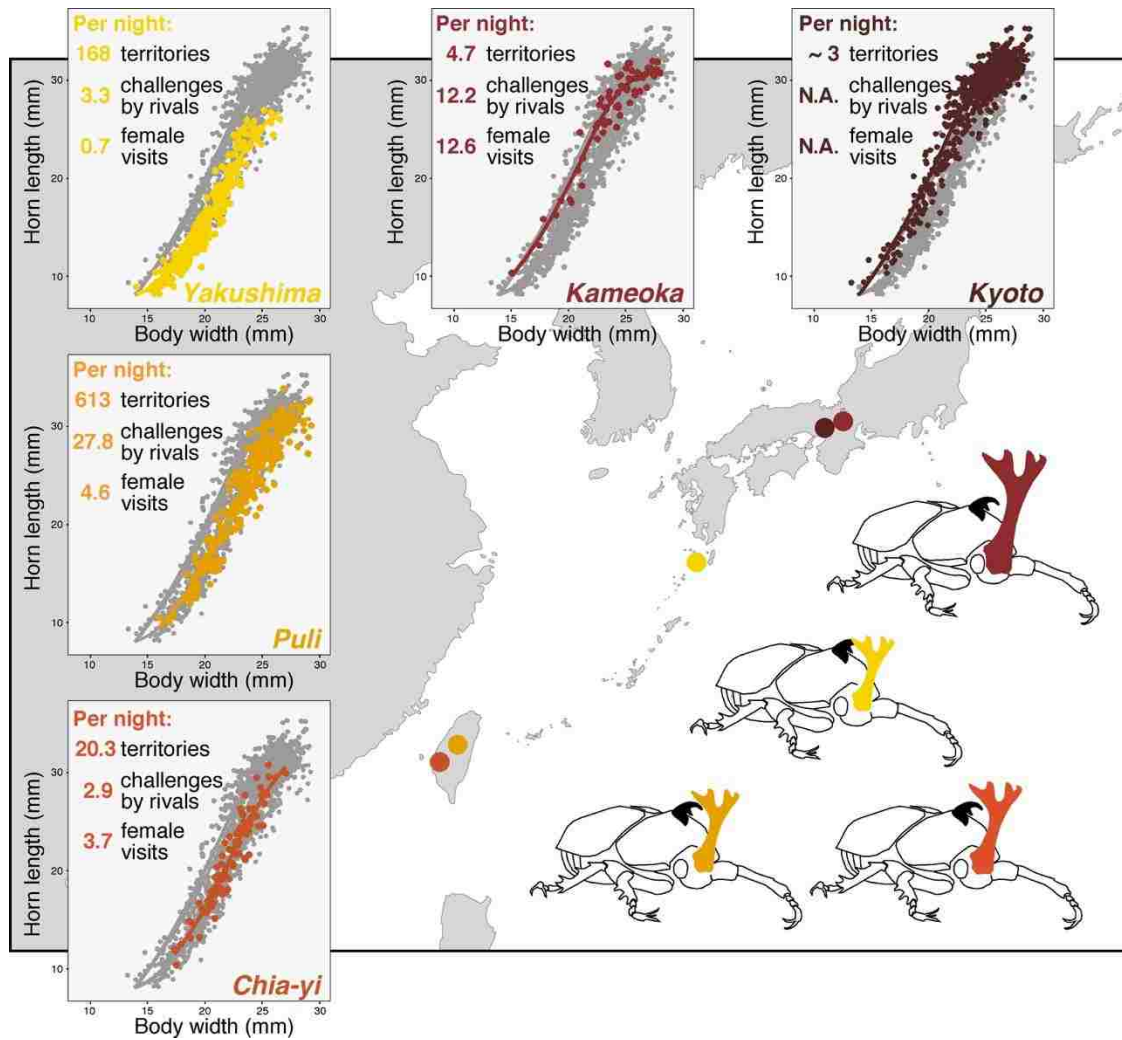
Figure 3: Locations, competitive conditions, and horn-body size scaling relationship of each of the 5 study populations.
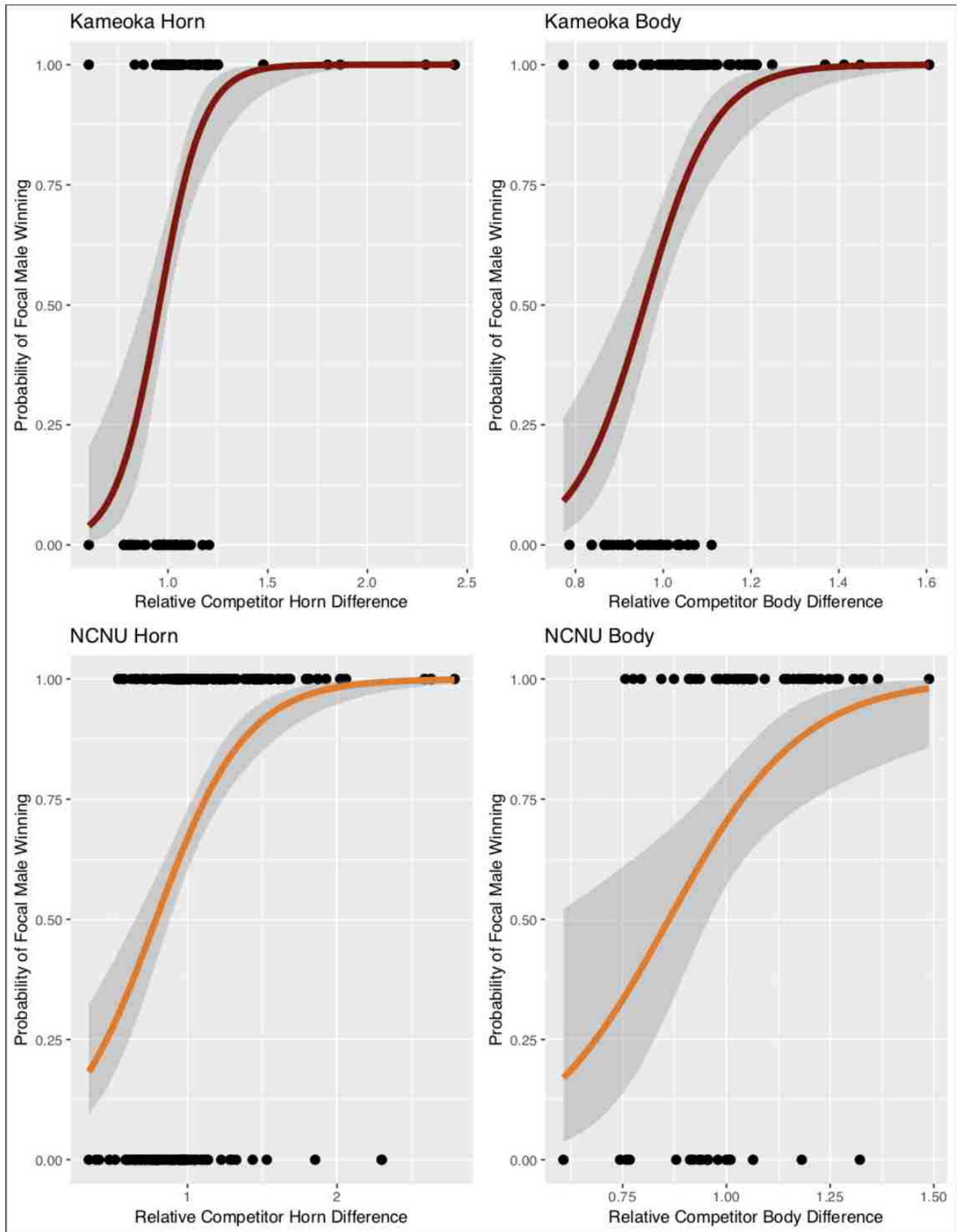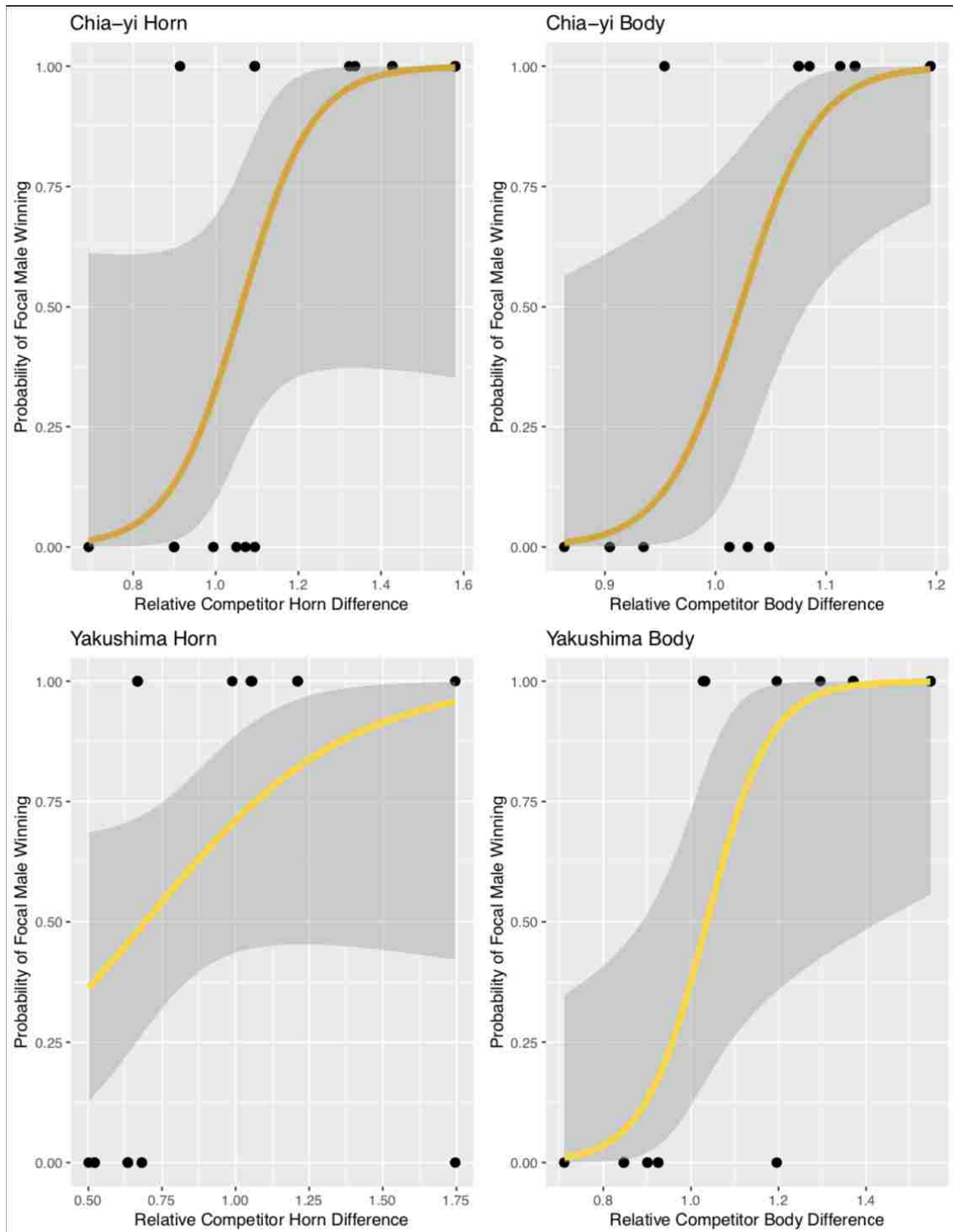
Figure 4: Logistic regressions showing the relationship between win probability and body size or horn size relative to opponent for Kameoka and the three short horned populations. In all populations, winners have larger weapon size and larger body size than their opponent.

Table 2: Models for Selection on Horns

Kyoto (data from Hongo 2007)

Model	Hypothesis	AIC	Parameters	Estimate (slope) ± SE	*p* value
Relative mate success ~ weapon size	I	2829.86	weapon size (*beta*)	0.43 ± 0.94	5.52 E-06
Relative mate success ~ weapon size + weapon size ^{2}	II	2809.81	weapon size (*beta*) weapon size (*gamma*)	10.75 ± 2.31 *10.90 ± 2.31	3.83 E-06 2.83 E-06
Relative mate success ~ weapon size + body size + weapon size*body size	III & IV	2798.13	weapon size (*beta*) body size (*beta*) weapon size * body size	0.146 ± 0.29 0.835 ± 0.25 0.351 ± 0.08	0.61 7.80 E-04 1.50 E-05
Relative mate success ~ residual weapon size	IV	2850.02	residual weapon size (*beta*)	0.05 ± 0.06	0.45
Relative mate success ~ residual weapon size + residual weapon size ^{2}	IV & V	2851.64	residual weapon size (*beta*) residual weapon size (*gamma*)	1.8 ± 2.39 1.46 ± 2.39	0.45 0.54
Relative mate success ~ |residual weapon size|	V	2850.46	weapon size (*beta*)	0.036 ± 0.10	0.71

Kameoka

Model	Hypothesis	AIC	Parameters	Estimate (slope) ± SE	*p* value
Relative mate success ~ weapon size	I	319.39	weapon size (*beta*)	0.55 ± 0.27	0.042
Relative mate success ~ weapon size + weapon size ^{2}	II	319.45	weapon size (*beta*) weapon size (*gamma*)	4.6 ± 2.2 *3.03 ± 2.2	0.041 0.175
Relative mate success ~ weapon size + body size + weapon size*body size	III & IV	321.14	weapon size (*beta*) body size (*beta*) weapon size * body size	0.63 ± 0.89 0.39 ± 0.76 0.31 ± 0.24	0.48 0.61 0.21
Relative mate success ~ residual weapon size	IV	323.50	residual weapon size (*beta*)	0.08 ± 0.19	0.69
Relative mate success ~ residual weapon size + residual weapon size ^{2}	IV & V	323.04	residual weapon size (*beta*) residual weapon size (*gamma*)	0.92 ± 2.27 -3.52 ± 2.27	0.68 0.13
Relative mate success ~ |residual weapon size|	V	320.29	weapon size (*beta*)	-0.52 ± 0.28	0.071

Table 2: Continued

Puli

Model	Hypothesis	AIC	Parameters	Estimate (slope) ± SE	*p* value
Relative mate success ~ weapon size	I	1091.23	weapon size (*beta*)	0.28 ± 0.14	0.041
Relative mate success ~ weapon size + weapon size ^{2}	II	1021.11	weapon size (*beta*)	4.26 ± 2.08	0.042
			weapon size (*gamma*)	*0.728 ± 2.08	0.73
Relative mate success ~ weapon size + body size + weapon size*body size	III & IV	1021.82	weapon size (*beta*)	-0.077 ± 0.42	0.852
			body size (*beta*)	0.41 ± 0.41	0.312
			weapon size * body size	0.067 ± 0.14	0.624
Relative mate success ~ residual weapon size	IV	1023.42	residual weapon size (*beta*)	0.011 ± 0.078	0.888
Relative mate success ~ residual weapon size + residual weapon size ^{2}	IV & V	1024.58	residual weapon size (*beta*)	0.295 ± 2.098	0.888
			residual weapon size (*gamma*)	1.915 ± 2.098	0.362
Relative mate success ~ |residual weapon size|	V	1022.81	weapon size (*beta*)	0.103 ± 0.131	0.43

Chia-yi

Model	Hypothesis	AIC	Parameters	Estimate (slope) ± SE	*p* value
Relative mate success ~ weapon size	I	395.966	weapon size (*beta*)	-0.340 ± 0.256	0.189
Relative mate success ~ weapon size + weapon size ^{2}	II	397.008	weapon size (*beta*)	-3.13 ± 2.36	0.19
			weapon size (*gamma*)	2.28 ± 2.36	0.34
Relative mate success ~ weapon size + body size + weapon size*body size	III & IV	397.36	weapon size (*beta*)	-1.23 ± 0.80	0.13
			body size (*beta*)	0.96 ± 0.81	0.24
			weapon size * body size	0.26 ± 0.221	0.24
Relative mate success ~ residual weapon size	IV	396.06	residual weapon size (*beta*)	-0.22 ± 0.173	0.20
Relative mate success ~ residual weapon size + residual weapon size ^{2}	IV & V	396.41	residual weapon size (*beta*)	-3.05 ± 2.36	0.21
			residual weapon size (*gamma*)	-2.99 ± 2.36	0.21
Relative mate success ~ |residual weapon size|	V	396.68	weapon size (*beta*)	-0.281 ± 0.27	0.31

Table 3: Net selection on weapon size

| Absolute horn lengh	Short-horned Populations		Long-horned Populations	
	Puli (Taiwan) 2016	**Chia-Yi (Taiwan) 2017**	**Kameoka (Honshu) 2017**	**Kyoto (Honshu) 2003, 2006**
Selection differential	1.078	-1.582	2.759	2.234
Standardized selection differential	0.045	-0.07	0.08	0.101
Selection gradient (*beta*)	0.28 ± 0.14	-0.340 ± 0.256	**0.55 ± 0.27**	**0.43 ± 0.94**

Figure 5: Male mating success in 4 populations as a function of their position on the population specific weapon-body size scaling relationship.

Supplementary Figures

Supplementary Figure 1: Horn and Pronotum Width Measurements
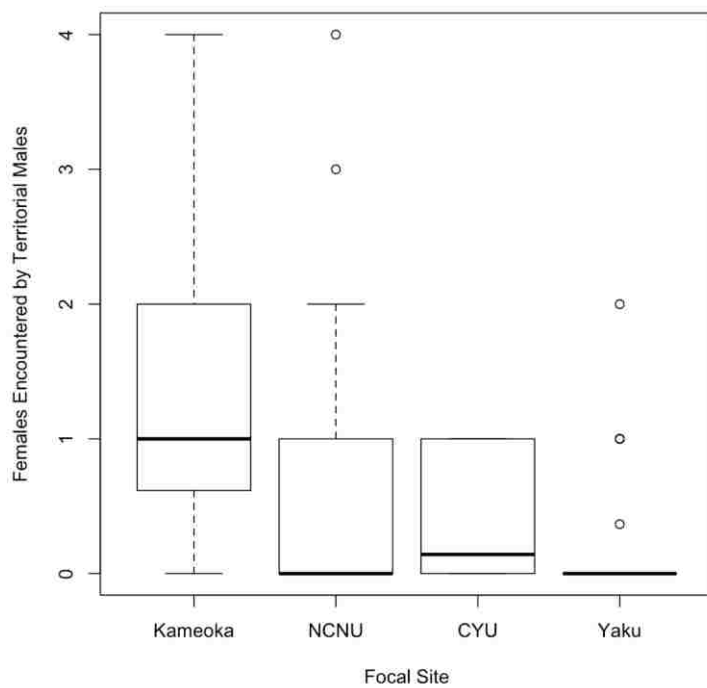
Supplementary Figure 2: Territorial males in Kameoka, the long horned population, encountered significantly more females per night than at each of the short horned populations.

Chapter 2

Contest Dynamics in Populations of Horn-Divergent Rhinoceros Beetles

Abstract

Weapons resulting from intrasexual selection are diverse, and they often evolve to extreme size. However, it is unclear whether the function of weapons in battle change along with their size (or vice versa). Specifically, exaggerated weapons are likely to serve a key signaling role in male-male competition, whereas their smaller counterparts may not. I tested these and other hypotheses put forth by contest theory, allowing me to quantify and compare the extent to which weapons serve as signals of fighting prowess in four populations of rhinoceros beetle that diverge in horn size and mating system ecology. Both weapon and body size influenced fight outcomes in all four populations. Differences in weapon and body size between opponents predicted the extent of fight escalation in three populations, suggesting that horns may be functioning as deterrent signals in these locations, but not at one of the short-horned locations. I used the relationship between fight duration and max escalation as a measure of the predictability of escalation of contests, or the degree of ritualization of contests, and found that the long horned population and the short horned population with the highest beetle density and most frequent fights, both had contests dynamics consistent with gradual escalation therefore male assessment. The remaining populations showed sudden and unpredictable escalation of fight intensity suggesting a lack of effective assessment of rival males, and presumably with a lack of a signal function of the male horns. Overall, my results suggest that beetles in populations experiencing the strongest sexual selection on weapon size, either due to resource limitation or high beetle density, incorporated assessment of rival males into fighting behavior, while populations with weaker net selection did not. This is the first such comparison of contest dynamics in a species with polymorphic weapons, and serves as an empirical bridge between the evolutionary subfields of contest theory and animal weapon evolution.

Introduction

Sexual selection via male competition has resulted in the evolution of diverse and exaggerated weaponry across the tree of life (Andersson 1994; Emlen 2008), but the selective and behavioral mechanisms underlying these patterns are still a mystery. Classic theory predicts that when the limited sex fights fiercely for access to rare mating opportunities, weapons can evolve to be larger as a result of increased reproductive benefits for well endowed contest winners, closely tying the size of animal weapons to the strength of selection determined by breeding success (Emlen 2008). However, selective environments are multifaceted, and weapons do not only function as tools. Many weapons serve dual functions: in addition to being used to pry, joust, or wrestle, weapons allow the bearers to communicate fighting prowess and guide a set of ritualized contest behaviors collectively known as contest dynamics (Parker 1964; Enquist and Leimar 1983; Berglund 1996). The development of contest theory allows us to quantify the nature and extent of male communication in physical encounters, and we can extend these concepts to predict how weapons might evolve ((Snell-Rood and Moczek 2013; Hardy and Briffa 2013; Painting et al. 2015).

Contest outcomes are typically determined not by death of the loser but by "limited warfare", a phenomenon where competitors avoid escalated and potentially injurious battle by first sizing each other up with less risky interactions like prodding or interlocking weapons. Game theory models of agonistic interactions predict that males will benefit by backing down from a confrontation if they can determine accurately that they are likely to lose, ending the fight before getting hurt (Maynard Smith and Parker 1973, 1976). However, males should only benefit from backing down when the information they glean regarding their opponent is a reliable predictor of the likely outcome of the contest (West-Eberhard 1983; Emlen and Nijhout 2000; Biernaskie 2014). Two factors in particular can affect the reliability of agonistic signaling.

First, accurate assessment of a rival is easiest if there is a conspicuous and honest signal of fighting ability that males can use to determine relative resource holding potential (RHP) of an opponent (Parker 1974; Enquist and Leimar 1983; Maynard Smith and Harper 1995). Exaggerated weapons of male competition often meet these criteria: they are large and highly visible, and they are often hypervariable in their expression from male to male. In addition, their growth tends to be unusually sensitive to variation in the nutritional state and/or physiological condition of the male at the time the weapon develops. Hyper- variability and 'heightened

conditional expression' (Cotton et al. 2004) cause these traits to be especially informative signals of male body condition and RHP, amplifying otherwise-subtle differences in condition, status, and/or body size so that they are especially easy to discern (Biernaskie et al. 2014). For this reason, males in many species use relative differences in weapon size to assess the RHP of an opponent (reviewed in Emlen 2008).

A second factor relevant to the efficacy of assessment concerns the nature of the fights themselves. The outcome of the contest must be highly repeatable, or predictable, before a male is likely to benefit from electing to back down, and not all contest outcomes are predictable (Hardy and Briffa 2013). Contests that unfold face-to-face between pairs of rival males are much more likely to unfold in ways that are repeatable than are contests involving 3 or more males (Emlen 2014). Indeed, one-on-one contests often unfold in ritualized, astonishingly repeatable sequences, as males escalate the intensity of the interaction in stages (Maynard Smith and Parker 1976). In these contests, it usually behooves males to pay attention to signals of the RHP of their opponent, collecting such information in stages as the fight proceeds in order to make an informed decision about when to back down from further injury (Enquist and Leimar 1983, Harper 2006). Such ritualized, dyadic encounters are the hallmark of contest theory, and these pairwise fights nearly always include males who opt to back down. Scrambles involving three or more males, on the other hand, are comparatively chaotic. In these contests the better fighter may not win, the benefits of large weapons may be less pronounced, and the unpredictability of the contest outcome means that males may elect to continue fighting even if they have the smaller RHP. Consequently, the social context in which the contests occur may influence the extent to which males assess their opponents, and thus the extent to which an exaggerated structure like a weapon functions as a deterrent signal, rather than a tool (McCullough et al. 2013).

In the present study, I observed male contests in the Japanese rhinoceros beetle *Trypoxylus dichotomus*, at four populations differing in relative weapon size, to explore the extent to which populations might differ in the relative importance of signaling in the function of the exaggerated male weapon. *Trypoxylus dichotomus* are a univoltine, sap-feeding rhinoceros beetle found across Asia. Their life cycle is confined to two summer months, and is relatively localized in suitable patches of broadleaf forest: both sexes congregate to feed at sap bearing trees, and females lay eggs in loamy forest soil before dying. Males battle with rival males for residency at these feeding territories, which are typically scarce, driving a resource-defense

mating system (Obata & Hidaka 1983; Siva-Jothy 1987; Hongo 2003, 2007). Recent work has shown, however, that the sap site resources are much less limiting in short-horned populations in Taiwan and southern Japan, suggesting an ecological driver of weapon divergence and the potential for shifts in the nature of local resource competition (del Sol et al. in prep).

Differences in resource value are known to shape the outcome of agonistic interactions and the nature of appraisal behavior (), but the extent to which this is true for the same species has never been examined. In addition, contest theory predicts that the higher degree of weapon exaggeration found in northern Japanese populations is consistent with a larger role of contest communication. One such population has already been shown to engage in ritualized appraisal behavior using the weapons (Hongo 2003), suggesting that shorter weapons in other populations may be a result of a reduced role of appraisal in male-male interactions over resources. In the present study, I quantified contest dynamics in the field in four distinct populations of *T. dichotomus*, three of which have relatively short horns (Puli and Chia-yi, Taiwan; Yakushima, Japan), and one with relatively longer horns (Kameoka, Japan). Specifically, I compare the patterns of escalation of fights (a measure of the extent of assessment) with the social context of fights (overall density of males, number of rivals in vicinity of fight, number of males involved in fights), and the relative length of the male horns in each population, to test population differences in the extent to which battles are resolved prior to escalation.

Methods and Materials

Study locations.

I conducted behavioral observations of four populations of *Trypoxylus dichotomus* over two summers comprising four, 1.5 month long breeding seasons in Taiwan and Japan, as part of another study (del Sol et al. in prep). In the summer of 2016, observations were conducted first on the forested campus of National Chi-Nan University, located in Puli, Taiwan (June-July). The second half of the summer (late July- August), I observed the full season on the island of Yakushima, Japan. In 2017, observations were conducted on the forested campus of Chia-yi University in Chia-yi Taiwan; in later Summer I observed beetles in a forested area in Kameoka, Japan.

Morphological measurements and scaling.

Observations began around 19:00 each night (prior to dusk and beetle emergence) in order to allow researchers to capture and measure unmarked males prior to their involvement in male-male interactions. Observations ended at sunrise, or otherwise when no pairs of male beetles could be observed in the same vicinity. For three populations, all actively sap-bearing trees were observed (NCNU, Kameoka, CYU); for the Yakushima, Japan population, we chose the most active 6 kilometer stretch of road and performed repeated surveys to confirm activity throughout the season.

Upon arrival at a tree, males were gently captured with a bamboo net or gloved hands, measured with dial calipers (Anytime Tools, CA, USA) and marked with a unique number on both elytra with non-toxic paint pen (Blick Art Supply, IL, USA). Repeated capture was avoided except to restore faded ID numbers. Horn length was recorded with one end of the calipers at the clypeal projection near the mouthparts, and the other end extended to the tip of the inner right horn projection to capture the full functional length of the structure (Supp. Fig. 1; Hongo 2007). Two measures of body size were recorded: elytral length (a straight line from the top of the scutellum to the bottom corner of the right elytron) and the maximum width of the pronotum (Supp. Fig. 1).

Contest Observations

Beetles are unable to see the color red (Enrodi 1992), so observations were conducted using headlamps with built-in red filters (RioRand, USA). Light beams were diffused and pointed adjacent to beetles to minimize disturbance, and were set to dim (200 lumens) whenever possible. One focal male at a time was chosen haphazardly to be observed for one hour. If the beetle was lost (flew or ran away, unable to be seen) after 30 minutes of observation, the observation was ended and the data were adjusted to account for time as per focal animal methods (Altmann 1974). During observation, each interaction with another male or female was noted. The duration of each behavior, including territory holding, fighting, and courting females, was recorded, as well as the number of beetles that arrived at a held territory. Territory quality was recorded as the number of females that approached or fed from a sap territory in an hour. I supplemented hour-long observations with intermittent sampling of aggregations, recording the details of additional fights through the course of the night.

For each male encounter, we recorded the ID numbers of each participant; the duration of the encounter; the maximum escalation score; whether a female was present; which beetle was the "owner" of the territory prior to the encounter, and which was the "intruder". Ownership was defined as active feeding, physical blockage of, or courting a feeding female at a territory. In several instances, ownership was defined as the participation in these activities more recently than the intruder. Duration of encounters was measured from the moment the territorial male "acknowledged" or faced the intruder to the point where the loser was either fully pried off the tree or ran at least 20 cm away from the winner. Encounters were virtually always instigated by physical contact from the intruder and elicited a startling response from the territorial male that allowed us to identify the beginning of an encounter for *both* participants.

Quantifying Escalation Stage.

Contest escalation is typically described by a species- specific set of behaviors that increase in intensity or contact and proceed in a predictable order as competitors gain more information about each other (Maynard-Smith 1974; Maynard Smith and Parker 1976; Enquist and Leimar 1983). For *T. dichotomus*, contest escalation has been described and quantified once before by Y. Hongo (2003). Since the final of the 6 stages only describes whether the loser flies or runs, I have compressed the contest observations into 5 separate stages that more strongly delineate the extent to which each competitors horn is used in the interaction. Observations were consistent in the order of these interactions, meaning that the final stage assigned to an interaction indicates that all prior stages happened as well.

Stage 1: Non-escalated. The males acknowledge interact, usually with a horn or leg touch, and acknowledge each other; then at least one male runs or walks away.

Stage 2: Non-escalated. Males acknowledge each other, then at least one male (possibly both) performs an aggressive gesture (i.e. not just turning towards the other beetle): "lunging" or horn jabbing, or chasing. One male leaves.

Stage 3: Non-escalated. Males engage in behaviors 1 and 2, and then one male pries the other male, who then ends the interaction by running away.

Stage 4: Escalated. Both males engage in behaviors 1-3; both males attempt to pry each other, but at different times. One male may run, then come back and pry. May end in the 'loser' running or being pried off the tree.

Stage 5: Escalated. A true battle. Behaviors 1-4, followed by at least one instance of mutual prying- defined as both participants have their horn under the other at the same time. Usually ends in a "de-escalation", or repetition of behaviors 1-4, in no particular order. May end in the 'loser' running or being pried off the tree.

Statistics.

All analyses were conducted in R 2.15 (R Core Team 2013). We used ANOVA to compare contest conditions between each population, including: hourly competitor density, territory density, number of opponents at a territory, and territory value (average number of females that approach a territory per hour).

Each male in an interaction was assigned a focal or non-focal status according to which male was the subject of focal observation during the encounter. Differences in competitor RHP can be assessed in multiple ways (Hardy and Briffa 2013); to capture a pairwise and population-independent metric, I calculated competitor difference as the ratio of focal RHP (horn or body size) to non-focal RHP.

Since my contest measures were not staged and conducted under field conditions, I accounted for repeated measures of individual males and of pairs by introducing both of these parameters as random effects in each model. Population was also included as a random effect in each model; upon significance, sites were analyzed separately. To test for the influence of horn size, body size, and relative horn size on contest outcome, we constructed a GLMM with a binary focal male won/opponent won response variable, and relative size difference as the predictor variable (horn and body size were run separately and together; Table 4. Due to the high collinearity of horn and body size (R~.92), only models with horn difference are reported). Additional models were run, one with territory ownership, and one with female presence, as fixed effects (Table 5.

We conducted several analyses to assess the adherence of contest behavior to classic predictions of contest theory in each population. In addition to the above predictors of RHP, we constructed a similar set of GLMM to assess the contributors to fight outcomes of only escalated fights to assess the likelihood that pre-escalation stages act as assessment behaviors in all populations (whereas it was found in a prior, long horned population; Hongo 2003). I ran logarithmic multiple regression models testing the probability of escalation based on the aforementioned size differences between opponents. I then ran multiple regression analyses testing for the relationship between duration and size difference. Further multiple regression between duration and the size of the loser or winner allows for the delineation of sequential assessment (SAM) and cumulative assessment (CAM) models of contest theory.

Results:

Competitive Environment and Territory Value

Overall, I conducted over 350 hours of focal male observation and observed 589 contests ranging across escalation stages (Kameoka, n = 123; Puli, n= 423; Chia-yi, n= 20 ; Yakushima, n= 23). Short-horned sites had more abundant territories and trees than long horned sites (Chaper 1, Table 1). Numbers of beetles were higher in Puli by an order of magnitude. The OSR was around 1.5 in Kameoka and Chia-yi, but was 3.4 in Puli and 5.0 in Yakushima. Fighting frequency was consistent with population numbers in short-horned populations, with territorial males in Puli battling, on average, 26 more times per night than in Chia-yi and Yakushima. Conversely, territorial males in Kameoka fought on average 12.2 battles a night despite having an average of 4.7 males on the tree at one time. Territory value, measured as female visitation numbers, was on average at least three times higher in Kameoka than in any short-horned population (Chapter 1, Table 1).

Predictors of contest outcome- weapons, body size, ownership, female presence

The initial GLMM showed no significance for individual and pair effects (p>0.1), allowing me to remove these parameters from subsequent models (those reported here; Table 4). Horn size was significant, suggesting that focal males with larger horns were more likely to win contests (Table 4). Site was also significant in this model (p<0.05); ANOVA revealed that the weapon size difference between competitors in Kameoka was significantly lower than the other

three sites (p < 0.05; Fig. 6). Logistic regressions showed that larger weapon size and larger body size significantly predicted the winner in every population (Chapter 1, Fig. 4).

Contest outcomes were influenced by territoriality and female presence (Table 5). Focal ownership of the territory was a significant predictor of focal wins (Logistic Regression, β= 2.084 ±0.19, p < 2e-16), while the presence of a female did not significantly affect the outcome of the contests (β= 0.211 ±0.23, p = 0.36). Site identity did not significantly affect either model (p > 0.1)

Escalation and Duration

Fights were more likely to escalate in Kameoka than in any short-horned site (ANOVA, df = 3, F = 27.73, p <0.001; Tukey Post hoc, p < 0.001). The likelihood of fight escalation was predicted by RHP (horn) difference in Kameoka and Puli, but not the other two short horned sites. (Fig. 6) The relationship between contest escalation and duration, a common metric of predictable contests (Briffa et al. 2013), was significantly positive in both Kameoka (OLS, p < .001) and Puli (OLS, p < .001). Longer contests did not escalate predictably in Yakushima (OLS, p > 0.1), and Chia-yi (OLS, P > 0.1).

Discussion

The tight link between environment and mating system characteristics means that sexual selection on weapons and other signals fluctuates across time and space, the latter being of particular interest in explaining the diversity of such traits (West-Eberhard 1983; Miller and Svensson 2014), While selection on weapons in general has been broadly characterized (reviewed in O'Brien et al. 2017), our understanding of the connection between competitive environment and animal weapon diversity is tenuous. There is some evidence that weapon diversity correlates with the diversity of fighting substrate (Emlen 2005; McCullough 2015) and general elements of the mating system like group size (Geist 1966; Bro-Jorgensen 2007). But local changes in social selection are likely the primary drivers of among-population differences in weapon form, and thus comprise the earliest stages of weapon divergence. A dearth of population-level comparative studies and the infancy of contest theory in general leaves us little to help predict the evolution of weapon and competitive behavior. One recent study suggests that

aggressive behaviors may be subject to phylogenetic inertia (Ayres-Peres et al. 2015), suggesting that behavior may change more slowly than local competitive conditions, with interesting consequences for mismatch and local selection on weapon form. In the present study, I have shown that while aggressive behavior in general may persist, contest environments and to some extent, dynamics are quite subject to shifts in mating system ecology.

Competitive Environment

Trypoxylus dichotomus is flexible in feeding, and is able to carve sap flows in thin-barked trees where its Japanese host tree *Quercus acutissima* is not found (Hongo 2006). This had notable effects in Puli and Chia-yi, where males and females were found to carve territories each night in the soft barked *Fraxinus* griffithii with the result that resources typically outnumbered males. Yakushima's native host *Machilus thunbergii* were abundant and each sported several cascading sap flows. In addition, we found remarkably few beetles each night, meaning that the resource defense system appeared to have collapsed entirely. Territorial males could expect to fight a number of competitors consistent with the abundance of males. Puli's population was large, and even though territories were abundant, males fought hard throughout the night to keep or gain sap wounds that had already been carved. Similarly, in Kameoka, though male abundance was relatively low, the whole population was confined to a single host tree, making competition intense *and* high reward. Whereas female abundance in Puli did not match male abundance, lowering the local territory value, Kameoka's dearth of sap flows meant that territorial males were more likely to be fighting for a chance to secure a mate than in any of the short-horned populations. While competition was fierce in two populations and diminished with lower population sizes in Chia-yi and Yakushima, Kameoka was the only place where territory value conferred the benefits of engaging in competition.

A note on mating system

Researchers of *Trypoxylus dichotomus* have long reported anecdotal evidence that females of the species only mate once (Siva-Jothy 1987, Hongo 2003, 2007), and a more recent study confirms this anomaly (Harada and Fujiyama 2017). Though rare (Eberhard 1996; Arnqvist and Nilsson 2000), systems with singly mating females suggest a mechanism that would maintain strong competition in spite of resource ecology. In the case of *T. dichotomus*,

females mated singly in all sites (del Sol et al. in prep), suggesting that territory limitation remains the deciding factor in competition dynamics and selection strength on weapons.

No Evidence for Female Defense or Scramble Competition

In a female-limited system, the presence of a female on a territory can reasonably be said to increase the immediate value of the territory. Thus, an opponent with such information is liable to adjust behavior accordingly (Enquist and Leimar 1987), and I predicted that males would adjust their behavior where territory value is low, as they have a lower chance of running into a female in a given night. However, fight outcomes were no different with females present, suggesting that male behavior remains in line with a purely resource-defense mating system in all populations despite the fact that, in short-horned populations, the competitive environment no longer constructs a beneficial relationship between competitive ability and mate success.

Residency effects are common in territorial species, as a territory holder has more RHP than an intruder by definition (Davies 1978; Grafen 1987). However, this may break down—either when territory value is low, like in Yakushima where females are incredibly rare—or when territory value is consistent across territories in systems like Taiwan, where feeding sites are made by males and females alike. This analysis also highlights the importance of conducting tests of behavioral and selective hypotheses with a keen eye towards natural history. Previous work in this system (del Sol et al. in prep) suggests that the primary driver of selection strength is feeding site, or territory, abundance. Males in short-horned populations can make their own territories; meaning that ownership may no longer be an accurate measure of RHP. Nevertheless, residency effects were consistent and pronounced across all sites, suggesting the consistency of a territory-defense mating system regardless of the breakdown of selection on competitive traits.

Contest Predictability

This study offers observations of wild populations with naturally occurring contests rather than staged. While this makes it difficult to tease apart the relative influence on weapon and body size, we are able to understand the nature of contests in a biologically relevant manner, allowing us to properly compare divergent populations. The nature of fights and of weapon size advantage was similar across all sites. Larger males with larger weapons were more likely to win contests, and this is expected from the consistency in the fighting style and fighting substrate.

Males pried and jousted with their horns, and males with longer horns reached their opponents first. Previous research shows that the horn is crucial in determining fight outcome (Karino et al. 2005), but this is the first demonstration of the consistency of horn use as a tool of battle in multiple, weapon- divergent population of this rhinoceros beetle.

In general, beetle competitive behavior was similar in all sites. Males viciously defended territories regardless of whether there were females present, or even likely to be present. Even though competitor densities were high in Kameoka and one short horned site, Puli, fights still invariably played out as duels. This served as a first quantifiable measure of predictability: the ritualized nature of battles (Enquist and Leimar 1983). I used the same escalation scale in each population, as higher levels of escalation did not occur without the preceding levels (n=523). Despite this, however, contest dynamics did partially break down at the two low-density sites, Chia-yi and Yakushima. At these sites, males tended to escalate fights independent of the magnitude of differences in RHP (Fig. 6).

As a final test of fight predictability, I examined the relationship between fight duration and escalation. If horns are being used as signals, then we should expect that more closely matched two opponents are, the more likely they should be to escalate in aggression and persist in battle as they gain information about each other (Briffa et al. 2013). Thus, a tighter relationship between these two fight characteristics indicates a more rigid adherence to the evolutionarily stable strategy of the war of attrition (Maynard Smith and Parker 1976). Using this metric, I found that three of the sites showed predictable escalation of contests, while one location, Yakushima, did not. While not completely illustrative of scramble competition behavior, this shift in contest dynamics at Yakushima indicates at least an early collapse of the resource defense system consistent with the fact that competitive ability and mating success are no longer connected at these two sites, due to low numbers of individuals and low territory value.

Ultimately, populations differed in the extent to which contests incorporated assessment, suggesting that the weapon may be functioning as a signal in some locations but not others. In one long-horned and one short-horned site, high competition was the result of limiting territories and high beetle density, respectively, but the ability to defend a territory translated into selective benefits most strongly in the long-horned population, as predicted. On several measures of predictability, the two short-horned populations with high territory number and lower density have collapsed, reflecting a predicted change in general contest dynamics as a result of the

competitive environment. In both populations, however, selection for horns was non-existent, suggesting that the behavioral changes were not, in fact, towards a use of the weapon that gave selective advantages. Rather, the shift in social environment (resource limitation) was likely sufficient to change both the selective pressure and fighting behavior in these populations in the short time since the populations' divergence (West-Eberhard 1983). Importantly, however, although key determinants of mating systems have diverged between these populations, the mating system itself has not. This provides some of the first evidence that weapons can change rapidly as a result of shifting selective benefits under the contemporary mating system, and not in response to immediate shifts in social and competitive environment. In predicting the drivers of weapon divergence and that of other sexual traits, this serves to illustrate the importance of constructing the link- quantitatively and as per natural history- between competition and mating success. Since the relationship between a male's success in battle and his mating success is ultimately what determines the selective benefits, prior trends of equating competitive environments to the strength of selection are likely insufficient to predict patterns of weapon divergence (Bro-Jorgensen 2007; Kokko et al. 2012). Future research in the contribution of sexual selection to the diversity of its products will require a keen eye both for the natural history of individual study systems and for the myriad ways in which these spectacular traits are used and perceived.

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Table 4: Results of binomial GLMM with focal male's horn size relative to opponent and site as fixed effects, individual id and fight ID as random effects; response variable is whether the focal male won.

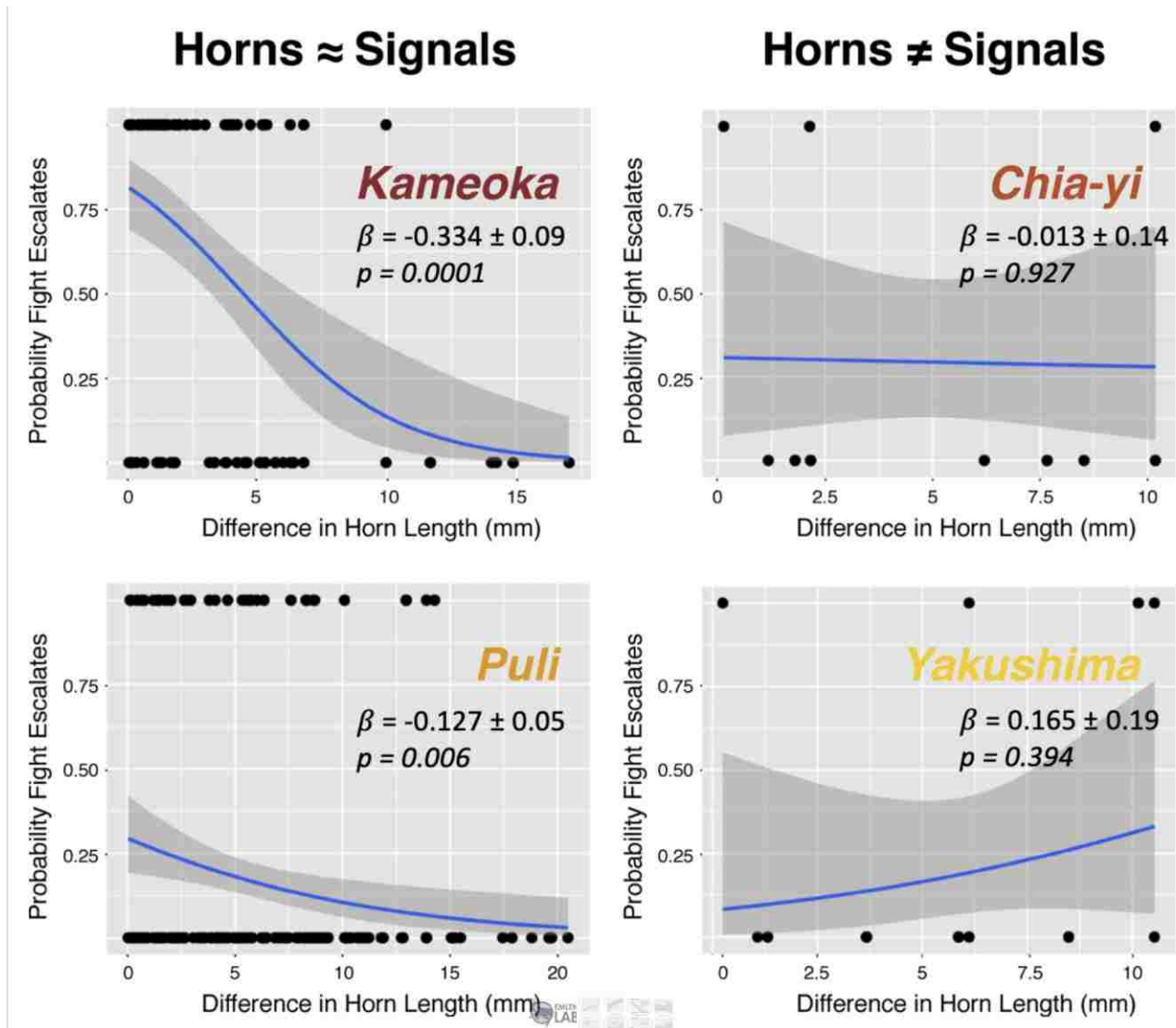| Model	Parameters	Estimate	SE	P-Value
Binomial GLMM; Response var = focal individ won (1/0); Individual ID and Pair ID as random effects	Intercept	1.1282	0.687	0.10
	Horn	1.9011	0.7619	0.01
	Site (Kameoka)	-1.9831	0.7502	0.0082
(individual and pair ID not significant; p<.1).	(other sites non-sig.; p< 0.1)			

Figure 6: The probability of encounter escalation based on the absolute difference in opponent's weapon size. Kameoka and Puli (A and C) show patterns consistent with the horn as a signal, with probability decreasing the larger the difference in opponent's RHP is. Chia-yi and Yakushima (B and D) show no significant pattern.
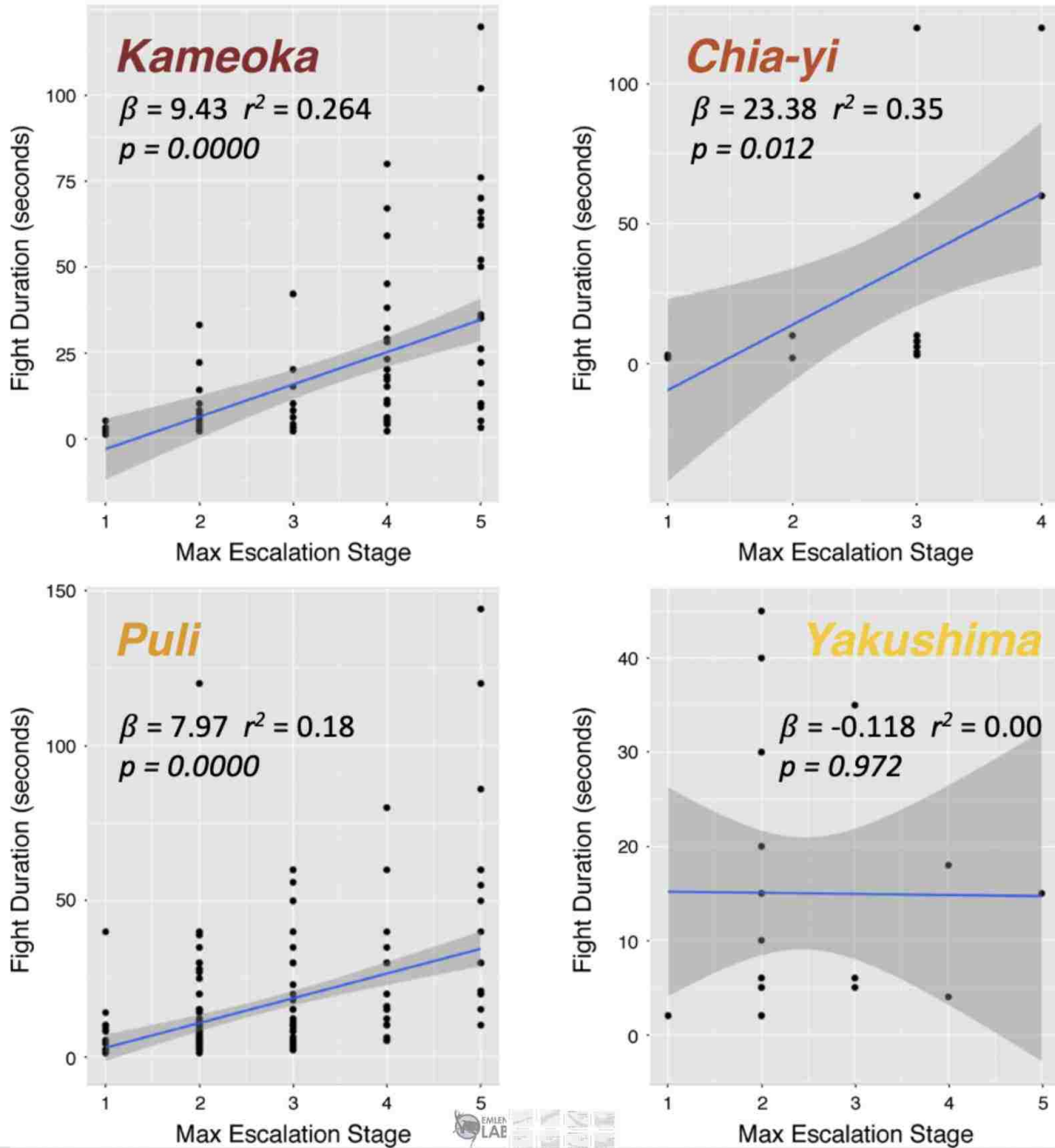
Figure 7: Contest escalation versus duration as a metric of fight predictability.