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Population differences in the strength of sexual selection match relative weapon size in the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae)

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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. We examined the ecology, reproductive behavior, and strength of sexual selection on horn length in five recently diverged rhinoceros beetle (*Trypoxylus dichotomus*) populations that differ in relative horn size. Males with longer horns were better at winning fights in all locations, but the link between winning fights and mating success differed such that selection favored large males with long horns at the two long-horned populations, but was relaxed or nonexistent at the populations with relatively shorter horns. Observations of local habitat conditions and breeding ecology point to shifts in the relative abundance of feeding territories as the most likely cause of population differences in selection on male weapon size in this species. Comparisons of ecological conditions and selection strength across populations offer critical first steps toward meaningfully linking mating system dynamics, selection patterns, and diversity in sexually selected traits.

KEY WORDS: animal weapons, male competition, mating systems, resource defense, sexual selection.

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; McCullough et al. 2016). Despite their shared function as tools of mate competition, weapons differ dramatically in shape, size, and position on the body between closely related taxa (Rosenberg 2002; Caro et al. 2003; Emlen et al. 2005a; 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 (e.g., West-Eberhard 1983; Kingsolver et al. 2001; McCullough et al. 2016), but the elaboration and diversification of these structures are not well understood.

Large-scale (e.g., family-level) comparative studies suggest that changes in the monopolizability of females (e.g., harem size) and fighting style (Kitchener 1991; Lundrigan 1996; Caro et al. 2003; Bro-Jørgensen 2007), and changes in the types of costs incurred from weapon expression (Emlen 2001; Emlen et al. 2005b), can drive evolutionary changes in weapon form; and biomechanical modeling suggests that changes in fighting style can drive changes in weapon form as well (McCullough et al. 2014; Klinkhamer et al. 2019).

Compared with ornaments, relatively few studies have quantified selection acting on weapons in the wild, and results to date

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vary (Conner 1988; Zeh et al. 1992; Wellborn 2000; Coltmann et al. 2002; Kruuk et al. 2002; Kelly 2004; Hongo 2007; Robinson et al. 2008; Vanpé et al. 2010; LeGrice et al. 2019; reviewed in O'Brien et al. 2017), with many studies failing to detect evidence of selection on weapons in contemporary populations (e.g., 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 (reviewed in O'Brien et al. 2017). If true, then 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 explore ecological factors responsible for driving the initial stages of weapon divergence (West-Eberhard 1983; Wellborn 2000; Tomkins and Brown 2004; Robinson et al. 2008; Cornwallis and Uller 2010; Miller and Svensson 2014; Buzatto et al. 2015; Toubiana and Khila 2019).

A few studies have compared patterns of selection across populations of the same species, and these suggest that changes in population density and resource availability each could drive fluctuations in the relative strength of selection acting on sexually selected weapons. In chorusing frogs (*Crinia georgiana*), males use enlarged forelimbs to grapple with rival males over egg-laying females, and selection often favors longer and stronger forelimbs. However, Buzatto et al. (2015) showed that local variation in the density of rival males affected the strength of sexual selection on male forelimbs: at low population densities, males could effectively monopolize females, and selection on forelimbs was strong; however, when population densities increased, multiple males piled on to females simultaneously and the strength of selection on forelimbs decreased (Buzatto et al. 2015).

Density of females, rather than rival males, influenced the strength of sexual selection acting on horn size in Soay sheep. In this case, males were better able to monopolize access to females when the number of fertile females was low, and the relationship between weapon size and mating success broke down when the numbers of receptive females increased (Preston et al. 2003). Selection on horn size was also affected by the overall abundance of foraging resources, because animals were able to overcome the costs of large horns when conditions were favorable, but had difficulty doing so when conditions were poor (in that case, viability selection ended up being more important than sexual selection; Robinson et al. 2008). Finally, an experimental evolution study of the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeinae) showed that the relative importance of pre- versus postcopulatory sexual selection changed with density, favoring large weapons more strongly when population densities (and the risk of sperm competition from sneak males) were low (McCullough et al. 2018).

Here, we examine the ecology, reproductive behavior, and strength of sexual selection acting on a forked head-horn weapon in five recently diverged populations of the Japanese rhinoceros beetle, *Trypoxylus dichotomus* (Coleoptera: Scarabaeidae; Dynastinae), which differ in relative horn length (Fig. 1). Specifically, we observed fighting and mating behavior at three locations with relatively short horns (National Chi-Nan University, Puli, Taiwan [June–July 2016]; Yakushima Island, Japan [late July–August 2016]; and Chia-yi, Taiwan [June–July 2017]), and one location with longer horns (Kameoka, Honshu, Japan [late-July–August 2017]), and we combined our findings with the results of 2 years' observations at an additional location with long horns, Kyoto, Honshu, Japan (July–August 2002, 2003; Hongo 2007).

Postcopulatory sexual selection is unlikely to be strong in this species, as females almost never remate (Siva-Jothy 1987; Hongo 2003, 2007, 2012; Harada and Fujiyama 2017), so we focused on patterns of premating sexual selection. In addition to comparing the relative strength of selection acting on horns in short- and long-horned populations, we provide preliminary evaluations of two putative ecological drivers of divergent sexual selection on relative horn length: horn length contributes more to winning fights in long-horned than in short-horned populations, and winning fights is more likely to translate into mating success in long-horned than in short-horned populations. We use our observations to propose an explicit scenario for how local changes in the distribution and relative abundance of feeding territories might be driving the initial stages of weapon divergence in this species.

Methods and Materials

STUDY SYSTEM

The Japanese rhinoceros beetle is a univoltine scarab found in broadleaf forests across Asia (Enrodi 1985). This species has been studied most extensively on Honshu Island, Japan, where adults emerge from the soil during summer months, fly to wounds on the sides of mature oak, ash, and maple trees (e.g., *Quercus mongolica*, *Quercus acutissima*, *Quercus serrata*, *Fraxinus griffithii*, and *Acer platanoides*; Hongo 2007), and feed on oozing sap (Siva-Jothy 1987; Setsuda et al. 1999; Hongo 2003, 2007). Males compete with rival males for residency at these feeding territories (Obata and 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 mating systems (Thornhill and Alcock 1983; Emlen 2008; Shuker and Simmons 2014). Fights between males are frequent, and males with relatively large body sizes and long horn lengths are most likely to win (Siva-Jothy 1987; Hongo 2003, 2007, 2012; Karino et al. 2005). Females mate with males at these feeding territories before leaving to lay

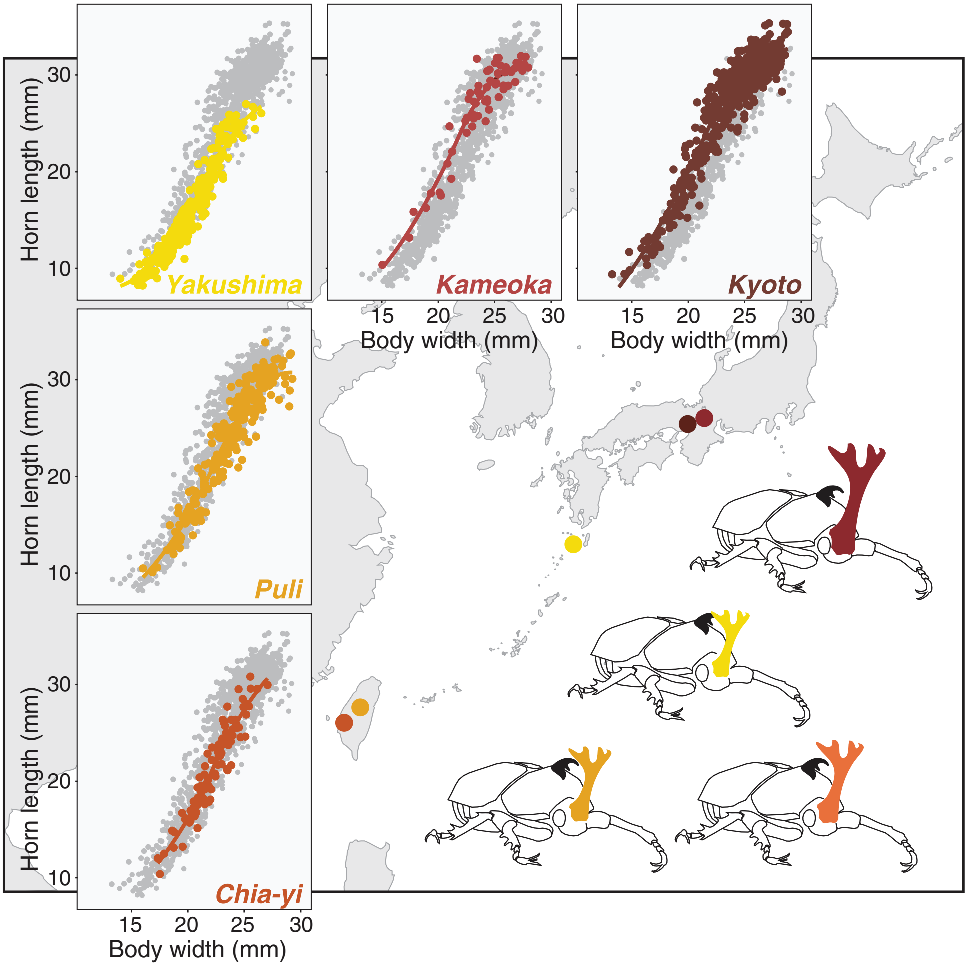

Figure 1. Location and relative horn lengths of each of the five study populations. Gray circles in each graph show the full range of horn lengths included in this study (i.e., all five populations combined).

eggs in decomposing litter up to a kilometer or more away (McCullough et al. 2012; McCullough 2013).

Like most sexually selected ornaments and weapons (Kodric-Brown and Brown 1984; Pomiankowski 1987; Zeh and Zeh 1988; Grafen 1990; Iwasa and Pomiankowski 1999; Cotton et al. 2004; Biernaskie et al. 2014), *Trypoxylus* horn size is condition dependent (Iguchi 1998; Fujiyama and Konno 1999; Karino et al. 2004, Plaistow et al. 2005; Emlen et al. 2012; Johns et al. 2014; Kojima 2015). Parent-offspring regressions for this species show no detectable heritable variation in horn length or body size (Karino et al. 2004). Instead, males modulate both weapon growth and body size in response to larval nutrition in a manner consistent with a developmental norm of reaction (Emlen and Nijhout 2000; Shingleton et al. 2007; Shingleton et al. 2008; Dreyer et al. 2016; O'Brien et al. 2017). Microevolution of *Trypoxylus* horns likely arises from genetic changes to these underlying nutrition- and condition-sensitive mechanisms (e.g., Emlen et al. 2007; Emlen et al. 2012; Ito et al. 2013; Adachi et al. 2018; Ohde et al. 2018; Zinna et al. 2018; Morita et al. 2019; Okada et al. 2019), which would be visible as population-level shifts in either the slope and/or the intercept of the scaling relationship between horn length and body size (Kawano 1995; Emlen and Nijhout 2000; McCullough et al. 2015). In this respect, *Trypoxylus* horn evolution is similar to the eyestalks of Diopsid flies (Wilkinson 1993; Baker and Wilkinson 2001), forceps of earwigs (Simmons and Tomkins 1996; Tomkins and Brown 2004), and mandibles of *Gnatocerus* flour beetles (Okada and Miyatake 2009) and Lucanid stag beetles (Huxley 1931; Otte 1979; Knell et al. 2004; Gotoh et al. 2012).

Populations included in this study differ in their respective scaling relationships such that males in three populations (Puli and Chia-yi, Taiwan, and Yakushima Island, Japan) have relatively shorter horn lengths than males in the remaining two (Kameoka and Kyoto, Honshu Island, Japan; Fig. 1). We use existing data collected in Kyoto between 2002 and 2006 (Hongo 2007) and season-long observations of male mating success, behavior, and ecology of the remaining four populations. The breeding seasons at all locations last approximately 1.5 months.

However, because Taiwan is located 7° latitude closer to the equator than the other locations, the breeding seasons of the two Taiwan populations started almost 2 months earlier than the other locations. This staggered onset of breeding permitted us 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 19:00h each night (prior to dusk and beetle emergence) to allow researchers to capture unmarked males upon their arrival at active trees prior to involvement in relevant behaviors. Males were gently captured with a net or gloved hands, quickly measured with dial calipers (Anytime Tools, Granada Hills, CA, USA), and marked with a unique number on both elytra with a nontoxic paint pen (Blick Art Supply, Highland Park, IL, USA). Horn length and pronotum width (a proxy for body size) were measured in accordance with Hongo (2007). Observations were conducted using headlamps with built-in red filters (RioRand, Richmond, BC, Canada). Light beams were turned to the lowest setting (200 lm) when possible and focused adjacent to beetles to minimize disturbance.

To compare the relative horn lengths of males in our study populations, we characterized the scaling relationships between horn length and body size. All statistical analyses in this study were performed using R (version 3.6.1; R Core Team 2019). For all models assuming normally distributed and homoscedastic errors, we systematically checked and confirmed this assumption by plotting the residuals against normal quantiles and residual versus fitted values. The scaling relationship between body and horn size has been previously recognized as nonlinear in *T. dichotomus* (Karino et al. 2004; Hongo 2007; Knell 2009; McCullough et al. 2015), so we followed the procedure highlighted in Knell (2009) to identify and characterize nonlinear allometries. Log-log scatterplots of residual versus fitted values from simple linear regressions revealed that horn length was systematically overestimated in large individuals, confirming a nonlinear, continuous relationship between horn length and body size in our populations.

For each population, we compared three models to describe this allometry (in log-log scale) using the Akaike's information criterion (AIC): (1) a simple linear model, (2) a quadratic model, and (3) a continuous breakpoint model (i.e., piecewise regression) separating individuals into major and minor males based on a body size threshold (Eberhard and Gutierrez 1991). For model 3, we determined the best single switch point and fit a continuous piecewise regression (*function*: "R package"; *segmented*: "segmented"; Vito and Muggeo 2008). The arbitrary starting value to find the best single breakpoint was set to the population mean of log10-transformed pronotum width. In contrast with the data for *T. dichotomus* analyzed in Knell 2009, the frequency histogram of the ratio of horn length to body size revealed a unimodal distribution in all of our study populations, precluding use of the Cook and Bean (2006) approach to separate minor and major males and fit a discontinuous model.

We chose the model with the lowest AIC to describe the horn length-body size allometry in each population. All populations had breakpoint allometries and were considered dimorphic with minor (<body size threshold) and major (>body size threshold) males except for Chia-yi, which had few males larger than the body-size break point. This population was subsequently treated as monomorphic (only minor males). Estimated breakpoints were compared between populations using 95% confidence intervals. Davies' tests were used in each population to test the difference in slope between the two segments—that is, between the two morphs—of the piecewise regression (*davies.test*: "segmented"; Davies 1987).

We tested for population differences in the slopes and intercepts of the scaling relationships of major and minor males using type I ANCOVAs and the linear model Log_{10}(horn length) $\sim \log_{10}$(pronotum width) × population × morph, and then performed pairwise post hoc comparisons of populations using Tukey Honest Significant Differences (*TukeyHSD*: "stats").

POPULATION DENSITY AND TERRITORY DISTRIBUTION AND ABUNDANCE

As with many beetles (e.g., Gries et al. 1994; Rochat et al. 2004; Wertheim 2005), adult *T. 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 across multiple breeding seasons, resulting in stable "hotspots" 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, we conducted hourly counts of the numbers of males and females arriving at territories, providing

Figure 2. Typical observational conditions and *Trypoxylus dichotomus* behaviors. (A) Two males fight over a feeding territory; the lower individual approached and challenged the territory-guarding male while he was courting a female (above and to the left). The female carves a new territory nearby and continues to feed while the males fight. (B) Successful mating attempts are easily recognizable because the male assumes a "hanging" position for 30–60 min while *in copula*. This characteristic posture made it possible to identify successful mating events even from a distance. Both trees shown are *Fraxinus griffithii* in Puli, Taiwan.

both nightly and cumulative estimates of overall beetle densities and local sex ratios. Because only reproductively active adults fly to feeding locations (Siva-Jothy 1987; Hongo 2003, 2007; McCullough et al. 2012; McCullough 2013), we estimated the operational sex ratio (OSR) as the average ratio of adult males to adult females at the breeding aggregations each night.

In addition to these population-wide measures, we conducted hour-long focal animal observations of males (Altmann 1974) to measure the frequency and type of interactions with competitors and potential mates. To compare the relative costs and reproductive benefits of guarding a territory at each of our study populations, we used the focal male observations to estimate the average number of approaches and/or challenges likely to be encountered by a guarding male during a typical night (a proxy for the price a male pays to hold on to a territory), and we estimated the average number of female visits to that same territory during a typical night (a proxy for the reproductive benefits of holding the territory).

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 min; Karino and 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, we surveyed all active trees every half hour throughout each night, locating every 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 each population across the entire breeding season.

Mating success is often a poor proxy for reproductive success, because numerous during- and postcopulatory processes can affect the likelihood that sperm transferred during a particular mating event end up fertilizing a female's eggs (Parker 1970; Eberhard 1996; Simmons 2001; Wedell et al. 2002). However, studies involving marked females in the wild suggest that multiple mating by *T. dichotomus* females is exceedingly rare (Siva-Jothy 1987; Hongo 2003, 2007, 2012). In the lab, females are reluctant to mate a second time even when housed with a second male for several weeks (e.g., in an experiment designed to measure sperm precedence from multiple mating; Harada and Fujiyama 2017), which is longer than the typical adult lifespan of beetles in the wild. Their reluctance to mate more than once, combined with a strong first-male sperm precedence (Harada and Fujiyama 2017), indicates that for this species mating success is likely to be an unusually rigorous proxy for fertilization success.

MALE HORN LENGTH AND FIGHT SUCCESS

We used our focal male observations to measure the outcome of male-male interactions at territories, and supplemented these samples with observations of additional fights collected intermittently throughout the season. The outcome of agonistic interactions was classified as 0 if the focal male lost the fight (i.e., retreated from the territory or got evicted by its opponent), or 1 if it won. For each fight, the focal male was designated as the owner of the territory if it was the resident male controlling the resource or the first present at the resource, or as the intruder otherwise. We combined our fight data from all locations into a single file (sensu Hardy and Briffa 2013) and ran generalized linear mixed models (GLMMs) using penalized quasi-likelihood (family: binomial, *glmmPQL*: "MASS"; Venables and Ripley 2002) to investigate the difference between populations in the effects of body size, horn length, and ownership on contest resolution, while avoiding pseudoreplication of fights involving common contestants (Hardy and Briffa 2013; Painting and Holwell 2014). Specifically, we ran the model: Fight Outcome ~ Pronotum Width + Horn Length + Ownership + Population + PW:HL + Population:PW + Population:HL + Population:Ownership, with fight ID and contestant ID included as random effects. We performed GLMMs using penalized quasi-likelihood because classic GLMMs (e.g., *glmer*: "lme4"; Bates et al. 2015) failed to converge on this complex model. All continuous variables were mean centered on zero and scaled to a unit variance. We report the significance of all fixed effects using a type III ANOVA (*Anova*: "car"; Fox and Weisberg 2019).

Post hoc pairwise comparisons using least square means and Tukey contrasts (*lsmeans*: "lsmeans"; Lenth 2016) were performed to further test how populations differed in the effect of ownership on fight outcome. For illustrative purposes, we built logistic regressions to plot the winning probability of the focal male as a function of a pronotum width or horn length difference index (DI) between the two contestants.

$$\mathrm{DI} = \left[\frac{\text{Size of larger contestant}}{\text{Size of smaller contestant}} - 1\right] \times 1 \text{ if focal male is larger,}$$

$$\mathrm{DI} = \left[\frac{\text{Size of larger contestant}}{\text{Size of smaller contestant}} - 1\right] \times -1 \text{ if focal male is smaller.}$$

This index has the advantages of being symmetrical around zero regardless of which contestant is larger.

SELECTION ON MALE HORN LENGTH IN THE WILD

We first compared the effects of body and horn size on male mating success across populations by using a generalized linear model. Most males did not achieve any copulations, whereas a few successful males obtained a relatively high number of matings, which resulted in a zero-inflated Poisson (ZIP) distribution of our mating success data. Thus, we built ZIP regressions (*zeroinfl*: "pscl"; Zeileis et al. 2008). ZIP models are indicated when count data display an excess of zeros; they assume that the excess of zeros is generated by a different process from the count values, and model them independently. ZIP models therefore include two parts: a Poisson count model and a logit (binomial) model for predicting excess zeros. We expected absolute mating success to be comparable between populations because the value of copulation is likely similar in each population as remating in females is extremely rare. For both the count and zero-inflation models, we initially ran the same formula: Absolute mating success ~ Pronotum Width + Horn Length + Population + PW:HL + Population:PW + Population:HL. We then went through a model selection process in both parts of the ZIP model based on AIC. We only report results from the best model (i.e., with the lowest AIC). Pronotum width and horn length were standardized (i.e., centered on zero and scaled to a unit variance). Significance of the fixed effects was assessed using type III Likelihood Ratio Tests (*lrtest*: "lmtest"; Zeileis and Hothorn 2002). Finally, we performed pairwise post hoc comparisons of population intercepts and slopes using Estimated Marginal Means with Tukey contrasts (*emmeans* and *emtrends*: "emmeans"; Lenth 2019). Significant interactions were also visualized using interaction plots (*emmip*: "emmeans").

In parallel, we also computed standardized selection differentials and gradients for each population (Lande and Arnold 1983; Brodie et al. 1995) using the Morrissey and Sakrejda (2013) method. This method is very similar to the classic Lande and Arnold (1983) approach in that it provides quantitatively meaningful selection gradients that have the same biological interpretation as the ones estimated by least squares linear regressions (Lande and Arnold 1983). However, it uses spline-based generalized additive models (GAM) to characterize the relationship between fitness and phenotypic traits and can model nonnormal distributions of fitness (Morrissey and Sakrejda 2013). First, we calculated directional standardized selection differentials (S) for body and horn size as the covariance between the relative mating success of an individual and the standardized trait (*moments.differentials*: "gsg"; Morrissey and Sakrejda 2014). Relative mating success for each individual was calculated as its absolute mating success divided by the mean absolute mating success in its population. We then built tensor product smooth-based GAMs (cubic regression spline with shrinkage) with a Poisson error distribution (*gam*: "mgcv"; Wood 2011) and absolute mating success as the response variable for each population. From these models, we calculated standardized linear (β) and nonlinear (γ) selection gradients for pronotum width and horn length for each population (*gam.gradient*: "gsg"). The standard error and the significance of the selection differentials and gradients were estimated after 10,000 bootstrap replicates.

Results

POPULATIONS DIFFER IN RELATIVE WEAPON SIZE

All of the studied populations had horn length-body size scaling relationships with the same basic curvilinear shape (Fig. S1A). Log-transformed, these relationships appeared as "broken lines" with minor males having a relatively steeper slope than major males (Fig. S1B; Tables S1 and S2). With the exception of Chia-yi, which had few males larger than the body-size break point and for which the breakpoint model was indistinguishable from a simple linear model, all of the relationships were significantly male dimorphic (Table S1). We therefore assigned males as majors or minors based on the estimated best-fit break points for body size (Eberhard and Gutierrez 1991; Knell 2009).

Long-horned populations (i.e., Kyoto and Kameoka) had significantly smaller body size breakpoints than Puli as attested by nonoverlapping 95% confidence intervals (Table S1). Davies' tests indicated that minor males had a significantly steeper slope than major males ($P < 0.0003$) for all the dimorphic populations ($P < 0.001$; Table S1; Fig. 1B). This difference was not significant in the Chia-yi population, which is consistent with the breakpoint model being undistinguishable from a simple linear model. The slopes of the horn length-body size relationships of minors and majors did not differ across populations (interaction: $\log_{10}$(Pronotum width):Population, $P = 0.69$; Tables S2 and S3). Minor males had hyperallometric allometries (i.e., slope > 1, disproportionate growth), whereas major males had isometric or hypoallometric allometries (i.e., slope = 1 or <1; Table S2).

The intercepts of the horn length-body size scaling relationships of both major and minor males differed significantly across populations (Population effect and interaction Morph:Population, $P < 0.0001$; Table S3), reflecting the observed population differences in relative horn length that originally motivated this study. Specifically, pairwise post hoc comparisons of these intercepts showed that males in both of the Honshu populations (Kyoto and Kameoka) had relative horn lengths that were significantly longer than the other three populations for both major and minor males (Chia-yi, Puli, Yakushima; $P < 0.0001$; Table S4). Horn lengths of males in Yakushima were also significantly shorter than those of males in Chia-yi and Puli, but these differences were very small (Fig. S1; Table S4). Consequently, for our subsequent comparisons of ecology and selection we classified our populations as relatively long horned (Kyoto and Kameoka) and short horned (Chia-yi, Puli, and Yakushima), respectively.

POPULATION DENSITY AND TERRITORY ABUNDANCE

Overall, the mating behavior of beetles was similar across locations. Adult males and females in all populations emerged from the leaf litter at dusk and flew to host trees to feed at active territories. Males encountered rival males on the sides of trees and battled over ownership of feeding 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 et al. 2012; McCullough 2013). 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 OSRs that were male biased (Table 1).

Although the general features of the mating system appeared similar at all study locations, some important details differed. First, beetles aggregated on different species of host tree. Beetles in both long-horned populations (Kyoto and Kameoka) fed on oak (*Quercus* spp.). Oak trees in these locations had tough, thick bark that was impenetrable to the chewing mouthparts of adult beetles, forcing the beetles to converge on existing sap flows created sporadically during summer months by burrowing Cossidae moth larvae (Hongo 2006, 2007; Yoshimoto and Nishida 2007). These sap flows were rare, typically concentrated on just a few individual trees in a given area, and they could stop flowing at any time if the moth larva stopped feeding. In the present study, a single oak tree hosted the entire breeding aggregation in Kameoka, with an average of 4.7 territories active on the trunk in a night; and in Kyoto (Hongo 2007), there were only seven suitable host trees, with an average of just three active territories per night (Table 1). As a result, in both of the studied locations with relatively long-horned beetles, territories suitable for *Trypoxylus* feeding were rare, concentrated on just a few individual host trees, and highly economically defensible.

Table 1. Mating system characteristics.

	Short-horned populations			Long-horned populations	
Site characteristics	Puli (Taiwan)2016	Chia-yi (Taiwan)2017	Yakushima Island2016	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
Number of host trees with active territories	212	26	117	1	7
Number of active territories per night	612.5 ± 51.9	20.3 ± 3.9	168.2 ± 3.6	4.7 ± 0.17	∼3
Number of males/females at breeding aggregation per night (OSR)	70.9/21.5 (3.3 ± 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)	16.5/8.3 (2.5 ± 1.9)
Territory characteristics					
Number of challenges by rival males per night	27.8 ± 4.25	2.9 ± 1.1	3.4 ± 0.9	12.2 ± 3.3	NA
Number of female visits to territory per night	4.6 ± 0.6	3.7 ± 0.7	0.7 ± 0.3	12.6 ± 1.2	NA

In contrast, there were no oak trees available at the Puli, Chia yi, or Yakushima locations. In both of the Taiwan populations (Puli and Chia-yi) beetles fed on ash (*Fraxinus griffithii*), and on Yakushima Island, Japan, they fed on bay (*Machilus thunbergii*). Ash and bay trees at these locations had much thinner bark than oak, and both male and female beetles could chew through the bark to create their own feeding territories. The fact that beetles could carve their own feeding territories meant that sap flows were far more abundant in short-horned populations than in the two long-horned populations (Hongo 2006; Ichiisi et al. 2019). Often, many dozens of suitable feeding territories were available to these breeding aggregations of beetles each night (Table 1).

The overall densities of beetles also differed dramatically across populations, with the Puli, Taiwan, population having the most animals (up to 300 beetles at the breeding aggregation per night and 900+ individuals throughout the season), and Yakushima and Kameoka having the fewest (approximately 11 and eight beetles at the aggregation per night, respectively; Table 1).

Consequently, both overall beetle densities and the relative limitation/abundance of critical feeding resources varied extensively from population to population. These factors contributed to population differences in the costs and benefits experienced by males attempting to guard territories. We used 350 h of focal animal observations of territorial males to estimate the average number of times each night that a guarding male faced challenges from rival males (a measure of the relative cost to a male of guarding a territory), as well as the average number of times each night that a guarded territory was visited by a female (a measure of the relative benefits of guarding a territory). We were able to estimate these "per territory" measures of the social environment for four of our study populations (Kameoka, Puli, Chia-yi, and Yakushima). However, these data were not collected in Hongo's (2007) original study, so we are not able to include Kyoto in this comparison.

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 3.3 ± 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 two populations (Yakushima and Kameoka) had very low overall numbers of beetles (approximately 11 and eight adult beetles per night, respectively). 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 beetle species (e.g., Howden and Campbell 1974; Gries et al. 1994; Hallett et al. 1995; Rochat et al. 2004; Morin et al. 2004). It also means that simply counting the number of beetles or available territories at a site is not sufficient to estimate the relative intensity of male competition and sexual selection.

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 eight adults per night), presumably reflecting the severe limitation of available territories at that site (Table 1).

Males guarding territories at the one measured long-horned population (Kameoka) were 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 benefits at locations where available feeding territories are most severely limiting (Table 1).

CONTEST DYNAMICS WERE 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 their horns to jab and lunge at the challenging male. In escalated fights, males attempted to use their forked head horn to pry opponents off of the tree, flinging them to the ground where possible. Despite pronounced differences in overall population density, and among-population variation in the species (oak, ash, and bay) and architecture (trunk diameter) of the host trees on which the beetles fought, battles in all locations unfolded as dyadic encounters between two rivals (i.e., duels). We did not observe a tendency for fights to devolve into multi-attacker scrambles at any of our studied populations, and in all locations males with the longest horns and largest body sizes were most likely to win (Fig. 3).

More specifically, male body size (pronotum width) had a significant positive effect on contest outcome across populations ($P = 0.046$; Fig. 3; Table 2). The effect of horn length, considered by itself, was not distinguishable from the effect of body size ($P = 0.64$; Table 2). However, there was a significant positive interaction between weapon and body size, indicating that large horns were more beneficial for contest outcome in larger males ($P = 0.01$; Table 2). Territory owners were also more likely to win contests ($P < 0.0001$) and populations differed in an interesting way in the relative effect of ownership on winning probability ($P = 0.012$; Table 2). Ownership mattered the most in our short-horned populations (i.e., Chia-yi, Yakushima, and Puli) compared with our long-horned population (i.e., Kameoka) ($P < 0.001$; Table 2). Other interactions were not significant

Table 2. Tests of the effect of body size, horn length, and ownership on contest outcome and population differences. A generalized linear mixed model using penalized quasi-likelihood (family = binomial) is presented here with Fight ID and Contestant ID included as random factors. Variables were standardized (mean = 0, unit variance). Only the pairwise contrasts between populations for owners are presented as none of the interpopulation contrasts were significant for intruders. Significant fixed effects ($P > 0.05$) are bolded.

Generalized linear mixed model (family: binomial)		ANOVA (type III)		
Variable	β ± SE	χ^2	df	*P*-value
Pronotum width	17.25 ± 8.82	3.98	1	**0.046**
Horn Length	–2.58 ± 5.65	0.212	1	0.64
Population	–	4.75	3	0.19
Ownership	Owner – intruder: 14.01 ± 3.56	16.08	1	**<0.0001**
Pronotum width: Horn length	1.60 ± 0.64	6.56	1	**0.01**
Population: pronotum width	–	4.95	3	0.18
Population: horn length	–	0.87	3	0.83
Population: Ownership	Post hoc tests: (Inter-population contrasts for owners) Chia-yi – Kameoka: 12.78 ± 2.76, **$P < 0.0001$** Chia-yi – Puli: 6.87 ± 2.69, $P = 0.06$ Chia-yi – Yakushima: –0.55 ± 3.78, $P = 1$ Kameoka – Puli: –5.92 ± 1.56, **$P = 0.001$** Kameoka – Yakushima: –13.33 ± 3.34, **$P = 0.0005$** Puli – Yakushima: –7.41 ± 3.24, $P = 0.11$	10.94	3	**0.012**

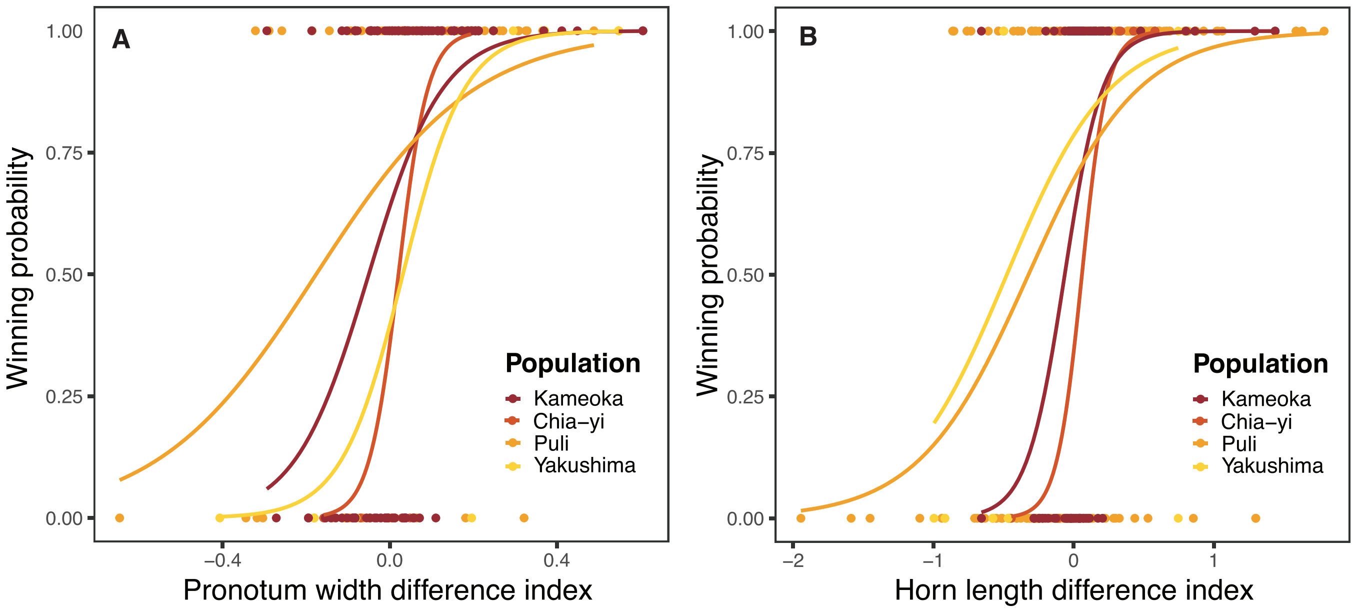

Figure 3. Logistic regressions showing the relationship between win probability and body size (A) or horn length (B) difference index between the two contestants for Kameoka, Chia yi, Puli, and Yakushima. Body size positively affected fight outcome in all locations, but the effect of relative horn size was more nuanced due to an interaction between horn length and body size: Horn length positively affected contest outcome preferentially in large males.

(Table 2), indicating that populations did not differ in other regards in terms of factors influencing fighting outcomes.

MATING SUCCESS AND SELECTION ON HORNS

Kyoto, Japan, had the largest sample of beetles ($N = 622$ males), and Chia-yi, Taiwan ($N = 86$ males), and Kameoka, Japan ($N = 71$ males), the smallest. Puli, Taiwan, had more animals than our two-person team could follow ($N = \sim300$ beetles per night). We still were able to census all successful copulation events with half-hourly surveys of the breeding aggregation, and between these males and the ones we could mark and follow, our included sample was 236 males. Yakushima, Japan, had 271 males but, despite conducting half-hourly censuses all night each night for the entire breeding season, we did not observe any mating events (possibly due to dispersed host trees and a very low nightly population densities; Table 1). As a result, we were not able to include Yakushima in the selection analyses.

We found that larger males had a higher lifetime mating success ($P = 0.003$; Table 3). The effect of horn size was indistinguishable from that of pronotum width ($P = 0.49$; Table 3). However, we found a significant and positive interaction between body and horn size: horns mattered the most for mating success in the largest males ($P = 0.008$; Table 3; Fig. S2). Interestingly, populations differed in the effect of horn size ($P = 0.001$; Table 3). Specifically, horn size mattered more for mating success in long-horn populations (i.e., Kyoto and Kameoka) than in short-horn populations (i.e., Puli and Chia-yi; Fig. S3). However, only the difference between Kyoto and Chia-yi showed significance ($P = 0.005$; Table 3). Similarly, we found that the probability of getting at least one copulation was influenced by body size ($P = 0.08$) and by the interaction between body and horn size ($P = 0.001$; Table 3). However, this time, we found a positive effect of horn length (even after accounting for body size) on this probability ($P = 0.04$; Table 3).

Using lifetime mating success as the measure of male fitness (Hongo 2003, 2007; Harada and Fujiyama 2017), we found significant positive linear selection differentials for both body and horn size in all populations except Chia-yi ($P < 0.05$; Table 4). Additionally, we found significant positive linear gradients for body size in long-horned populations (i.e., Kyoto and Kameoka; $P < 0.05$) but not in short-horned populations (i.e., Puli and Chia-yi) ($P > 0.1$, Table 4). The linear selection gradient for horn length was only significant for the Kyoto population ($P < 0.01$, Table 4). Overall, the linear gradients for body and horn size were higher in long-horned populations, with even negative gradients in the Chia-yi population. Finally, we found significant positive quadratic gradients for body and horn size ($P < 0.1$), along with a significant positive correlational gradient between body and horn size, in the Kyoto population ($P < 0.001$; Table 4). Consistently, mating success landscapes showed a striking difference between long- and short-horn populations, notably between Kyoto and Puli (Fig. 4), and illustrate well that large horns in large individuals matter much more in the long-horn populations. These landscapes are much shallower and flatter in

Table 3. Statistical results from the best zero-inflated Poisson (ZIP) regression model testing the effects of body size, horn size, and their interaction on absolute male mating success and the differences between populations. Both parts of the ZIP model—i.e., the Poisson count model and the logit model for predicting excess zeros—are presented. Continuous explanatory variables were centered on zero and scaled to a unit variance. Post hoc pairwise comparisons using estimated marginal means and Tukey contrasts (functions *emmeans* or *emtrends* in R package "emmeans") are reported for categorical variables. Significant effects are bolded ($P < 0.05$), as determined by type III likelihood ratio tests.

	Zero-inflated Poisson regression		Likelihood ratio tests	
	Explanatory variable	Estimate ±	χ^2	*P*-value
Count model	Pronotum width	0.72 ± 0.23	8.81	**0.003**
	Horn length	0.19 ± 0.27	0.47	0.49
	Population	Post hoc tests (emmeans): Kyoto – Chia-yi: 0.11 ± 0.08, *P* = 0.45 Kyoto – Kameoka: 0.15 ± 0.07, *P* = 0.19 Kyoto – Puli: 0.14 ± 0.05, ***P* = 0.01** Chia-yi – Kameoka: 0.03 ± 0.09, *P* = 0.98 Chia-yi – Puli: 0.03 ± 0.07, *P* = 0.98 Kameoka – Puli: -0.009 ± 0.07, p = 1	12.94	**0.005**
	Pronotum: Horn	0.20 ± 0.07	6.97	**0.008**
	Population: Horn	Post hoc tests (emtrends): Kyoto – Chia-yi: 0.92 ± 0.28**, *P* = 0.005** Kyoto – Kameoka: –2.28 ± 1.45, *P* = 0.39 Kyoto – Puli: 0.51 ± 0.26, *P* = 0.21 Chia-yi – Kameoka: –3.20 ± 1.50, *P* = 0.14 Chia-yi – Puli: –0.41 ± 0.21, *P* = 0.21 Kameoka – Puli: 2.79 ± 1.51, *P* = 0.25	15.53	**0.001**
Zero-inflation model	Pronotum width	1.39 ± 0.88	3.10	0.08
	Horn length	1.69 ± 0.94	4.07	**0.04**
	Pronotum: Horn	–2.35 ± 0.96	10.16	**0.001**

Table 4. Standardized selection differentials (S), linear selection gradients (β), and matrix of quadratic and standardized selection gradients (γ) (± Standard Error) on male pronotum width and horn length, based on mating success.

Kyoto ($N = 622$)	S ± SE	β ± SE	Pronotum width	Horn length
Pronotum width	**0.543 ± 0.082*****	**0.931 ± 0.144*****	**1.107 ± 0.283*****	
Horn length	**0.431 ± 0.073*****	**0.513 ± 0.185****	**1.487 ± 0.305*****	**1.035 ± 0.510**$^{+}$
Kameoka ($N = 71$)	S ± SE	β ± SE	Pronotum width	Horn length
Pronotum width	**0.578 ± 0.140****	**0.562 ± 0.267***	–1.302 ± 1.251	
Horn length	**0.544 ± 0.124****	1.476 ± 0.882	1.241 ± 0.824	2.879 ± 2.894
Puli ($N = 236$)	S ± SE	β ± SE	Pronotum width	Horn length
Pronotum width	**0.310 ± 0.132***	0.270 ± 0.162	0.228 ± 0.221	
Horn length	**0.277 ± 0.127***	0.193 ± 0.203	0.303 ± 0.194	0.105 ± 0.108
Chia-yi ($N = 86$)	S ± SE	β ± SE	Pronotum width	Horn length
Pronotum width	–0.233 ± 0.280	–0.007 ± 0.138	0.091 ± 0.136	
Horn length	–0.336 ± 0.261	–0.196 ± 0.141	0.115 ± 0.127	0.061 ± 0.050

Diagonal elements in the γ matrix correspond to the quadratic selection gradients and the off-diagonal element to the correlational selection gradient.

Significant differentials and gradients are bolded.

$^{+}P < 0.1$;

$^{*}P < 0.05$;

$^{**}P < 0.01$;

$^{***}P < 0.001$.

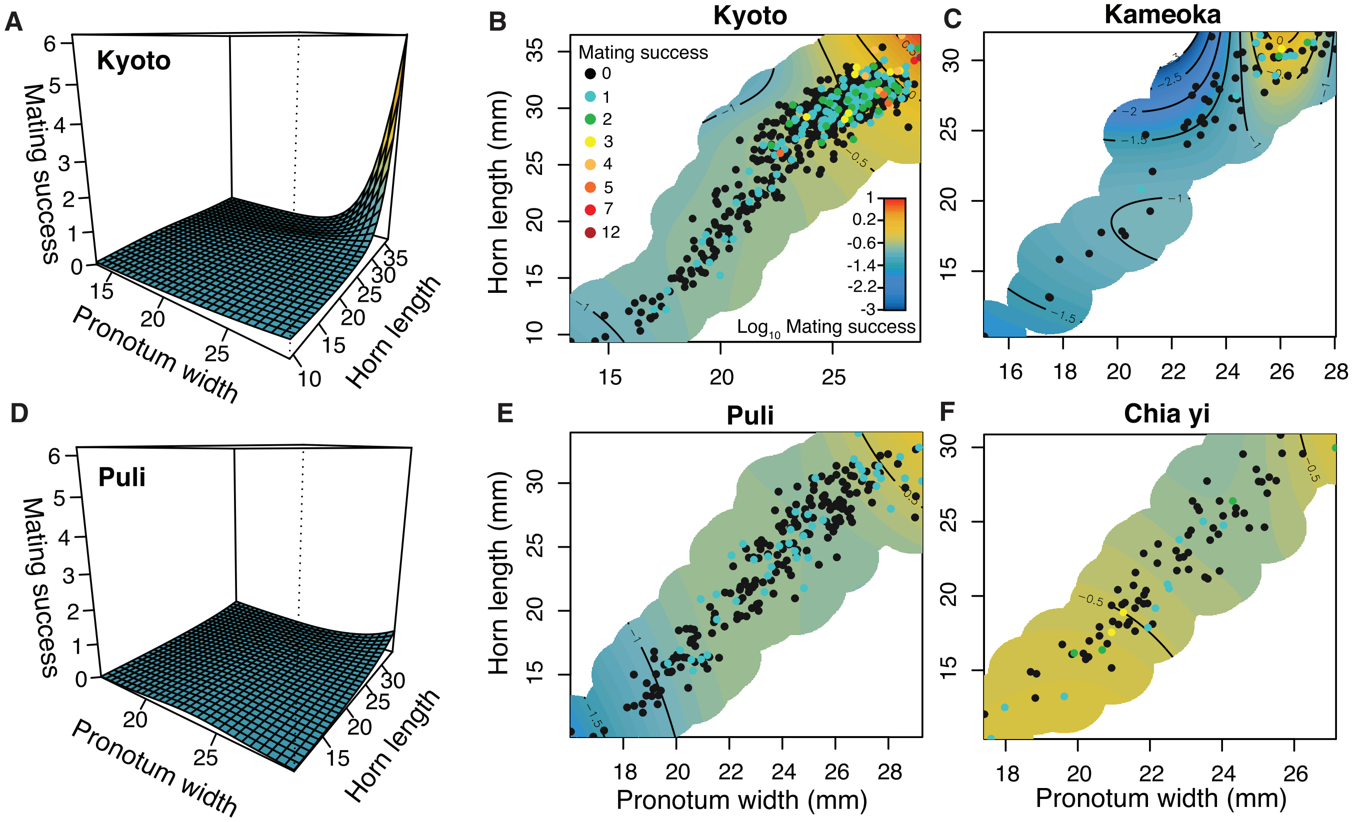

Figure 4. Male mating success in long- (A–C) and short- (D–F) horned populations. Panels A and D show male absolute mating success landscapes as a function of pronotum width and horn length for the two populations with the largest sample sizes (Kyoto: 622; Puli: 236). Panels B, C, E, and F show raw male mating success in four populations as a function of their position on the population-specific weapon-body size scaling relationship. Background colors and contour lines show the local topography of the log-transformed mating success landscape. Legends and scales are presented in panel B and consistent for all populations (B, C, E, and F).

the short-horn populations, consistent with the smaller selection gradients.

Discussion

Our samples confirmed that populations of *Trypoxylus dichotomus* differ in relative weapon size. Both of the Taiwan populations (Puli and Chia-yi), and the island of Yakushima, had beetles with relatively shorter horn lengths than were observed in the two Honshu, Japan, populations (Kyoto and Kameoka), and these differences resulted primarily from shifts in the intercept of the scaling relationship between horn length and body size (Fig. S1; Tables S1–S4). Population differences in relative horn length persist in captive populations of professional breeders, and the populations included in this study are formally recognized as subspecies by systematists (*Trypoxylus dichotomus septentrionalis* [Honshu], *T. d. shizuae* [Yakushima], *T. d. tsunobosonus* [Taiwan]; Kono 1931; Kurosawa 1985; Adachi 2017), suggesting that the differences we observe are genetic. Although common garden experiments will be needed to fully resolve this issue, we treat these population differences as recently evolved shifts in relative weapon size.

No female mated with more than a single male in any of our observed populations, confirming earlier suggestions that female *T. dichotomus* mate just once in their lifetime (Siva-Jothy 1987; Hongo 2003, 2007, 2012; Harada and Fujiyama 2017). As a result, we considered the effects of during- and postcopulatory sexual selection to be minimal in this species, and focused instead on relative mating success of males. We were able to estimate the lifetime mating success of more than 1000 male beetles and overall, selection on both body size and horn length was positive and significant (Table 3). We also observed a significant positive interaction between the effects of horn length and body size. Specifically, large males benefitted from having long horns, whereas intermediate-sized and smaller males did not (Fig. S2; Table 3). Finally, populations differed in the relative strength of sexual selection on horn length, with horn size mattering more in long-horned (Kyoto and Kameoka) than in shorter-horned (Puli and Chia-yi) populations (Fig. S3).

Why might populations differ in the intensity of contemporary sexual selection acting on weapon size? Although it was not a primary aim of this article, we were able to collect detailed information on the natural history and breeding ecology of beetles at each location, and these data are sufficient to begin to address two alternative hypotheses for the ecological drivers of local differences in the strength of sexual selection: (1) horn length contributes more to fight success in long-horned than in short-horned populations and (2) winning fights translates more directly into mating success in long-horned than in short-horned populations.

If the details of male battles differ from location to location, then long horns might confer a greater fight performance advantage in some populations than in others. Most studies of male weapons quantify the effects of weapon size on fight performance using staged contests (e.g., Emlen 1997; Sneddon et al. 1997; Moczek and Emlen 2000; Karino et al. 2005; Kelly 2006; Judge and Bonanno 2008; Yasuda et al. 2012; Walker and Holwell 2018). By pairing same-sized opponents with different weapon sizes, this approach can separate contributions of the weapon from the often-confounding effects of body size (Arnott and Elwood 2009; Briffa et al. 2013; Hardy and Briffa 2013). Staged fights consistently demonstrate that the male with the longer/larger weapon is most likely to win. But staged contests are also always set up as *duels*—dyadic encounters between two opponents. The outcome of duels is highly predictable, and in these fights it is not surprising that the larger, better-armed contestant usually wins.

Not all male-male contests occur as duels, however. Contests in many animals involve simultaneous attacks by multiple males. The outcomes of these scrambles can be far less predictable than that of duels (Parker et al. 2013; Emlen 2014a, b;Buzatto et al. 2015; Heberstein et al. 2017), decreasing the benefits of investing in costly weapons (Parker et al. 2013). Indeed, species that typically face scrambles almost never have large weapons (reviewed in Emlen 2014a, b;Heberstein et al. 2017). We hypothesized that this same logic might also help explain among-population variation in relative weapon size (e.g., Buzatto et al. 2015). Specifically, if fights in short-horned populations were more likely to devolve into scrambles, forcing territorial males to face multiple challengers simultaneously (sensu Emlen and Oring 1977; Kokko and Rankin 2006; Knell 2009), then the benefits of long horns might be reduced in these populations resulting in weaker net selection on horn length.

This is not what we observed. All fights in all locations occurred as one-on-one duels, and in all of our study populations males with longer weapons and larger body sizes were consistently likely to win (Fig. 3; Table 2). Consequently, local differences in the fights themselves do not appear to be contributing to population differences in the relative strength of selection on horn length.

Our field observations did provide preliminary support for the second hypothesis, however. Male-male competition is predicted to drive the evolution of large weapons when the best-armed males are able to monopolize disproportionate access to reproductive females. Selection drives the evolution of large weapons because winning fights translates into winning opportunities to mate. Several ecological factors may influence the strength of this link between fighting and mating success (e.g., Emlen and Oring 1977; Blanckenhorn et al. 1999; Jann et al. 2000; Pröhl 2002; Klug et al. 2010; Parker et al. 2013; Miller and Svensson 2014). For example, high population densities might create opportunities for sneak males to intercept females while their rivals are distracted in battle, reducing the mating benefits of winning fights. Alternatively, population differences in the distribution and /or abundance of guarded resources might cause females to be more likely to visit territories in some populations than in others. In both situations, local differences in the social and/or physical environment could affect the link between fighting and mating success, and so alter the relative intensity of selection acting on weapon size.

Here, we measured both fighting success and lifetime mating success of males in the wild, allowing us to examine this critical link between winning fights and winning mates. Although long horns aided males in winning fights in all of our studied populations, the extent to which fighting success translated into mating success differed. In our two long-horned study populations (Kyoto and Kameoka), long-horned males not only won fights but also were the most successful at mating with females (Fig. 4; Table 3). In contrast, in the two short-horned populations for which we have mating success data (Chia-yi and Puli), reproductive success was more uniformly distributed across the weapon and body size spectrum, and the most successful males were not the largest or longest horned (Fig. 4; Table 3). Males in these populations still battled for access to feeding territories, and males with the longest horns and largest body sizes still won. However, winning fights failed to translate into disproportionate access to mating opportunities and, as a result, the reproductive benefits males gleaned from defending territories disappeared. Consequently, population differences in the strength of the link between winning fights and winning mates corresponded with local differences in the relative intensity of sexual selection acting on weapon size.

Interestingly, two of the most common proxies for sexual selection, the OSR and population density, were not good predictors of the strength of selection acting on horns in our study populations. Klug et al., (2010) caution that when males are unable to monopolize access to females, the OSR may not be a useful predictor of the intensity of sexual selection, and this is what we observe in *Trypoxylus*. In each of our short-horned populations, available feeding territories were abundant and widely distributed across host trees. Because of this, females were less likely to visit any particular territory. The potential for males to monopolize access to females was poor, and in these populations the intensity of sexual selection on horns was relaxed despite the OSR being strongly male biased (e.g., 3.3:1 in Puli).

Population density is also often used as a proxy for the intensity of sexual selection, although the relationship is less linear (e.g., both very high and very low densities of rival males can result in weaker sexual selection; Thornhill and Alcock 1983; Shuker and Simmons 2014). Here, our most dense population (Puli) had no detectable selection on weapon size ($\beta = 0.193 \pm 0.203$; Figs. 4D and 4E; Table 4), not because males were fighting so often that they failed to mate or because they were unable to effectively guard territories due to multiple simultaneous attackers; but simply because there were so many feeding territories that females were widely dispersed and unlikely to visit a guarding male. This eroded the reproductive benefits males gleaned from fighting to guard a territory and relaxed the intensity of net sexual selection on horn length, even though males were using their horns to advantage in several dozen fights each night. At the other extreme, our lowest density population (Kameoka, average four males and three females per night) still experienced positive selection for long horns ($\beta = 1.476 \pm 0.882$; Fig. 4C; Table 4), presumably due to severe limitation of available feeding territories and high probabilities of nightly visits by females (12.6 ± 1.2 visits per territory each night; Table 1).

Conclusions

Our field measures of selection tracked closely with population differences in relative horn length of *Trypoxylus* beetles. Specifically, the two populations with relatively long horns (Kyoto and Kameoka) experienced positive selection for longer horns in the largest males, whereas selection in populations with relatively short horns was absent. Overall, the population-level patterns of selection we observe in *Trypoxylus* are compelling, and although snapshots of contemporary selection are never sufficient to demonstrate causality, these patterns do point to an explicit hypothesis for the ecological drivers of horn evolution in this species.

On the Asian mainland and the large islands of Japan (e.g., Honshu and Kyushu), beetles congregate on a small number of feeding territories created on the trunks of oak trees by the excavations of other species (e.g., the larvae of a moth). These sap flows are rare and males successful at guarding them are disproportionately successful at encountering and mating with females, who must visit these sites to feed before laying eggs (Hongo 2007; this study). The link between fighting and mating success is strong in these populations, and the result is positive selection for long horns.

When beetles colonized the offshore islands of Taiwan and Yakushima, however, their preferred host tree was not available. These beetles appear to have shifted to feeding on sap of a new host tree (ash on Taiwan; bay on Yakushima). Both ash and bay trees have thinner bark than oak, and beetles on these islands could now carve their own feeding territories. Although the overall behavior and mating system remained largely intact at these locations, the relative abundance of feeding territories increased (e.g., Puli, with more than 600 active feeding territories). Males still fought to guard wounds on the sides of trees, and long horns still aided them in these battles. However, the increased abundance of feeding territories, and their wide distribution over more of the trunk surface of a greater number of host trees, resulted in a lower probability that any particular feeding territory would be visited by a female. This simple shift in the relative abundance and dispersion of feeding territories, we suggest, eroded the reproductive payoffs of guarding males in these populations, relaxing selection on male horn length and contributing to the evolution of relatively shorter horns.

It is interesting that despite an abundance of feeding territories at these island locations, and despite very low population densities at two of them, male beetles still approached and challenged each other, and feeding males still fought to guard their feeding sites from these rivals. As a result, males continued to use their horns—and the longest-horned males continued to win—despite what appears to be a collapse of the mating system occurring around them. The biggest males still pay the developmental and maintenance costs of producing and wielding their weapons, and they still expend energy in repeated contests each night, even though these same males are no longer the most likely to mate. The essential, and all-too-often assumed, link between fighting and mating success has eroded in these populations, yielding relaxed net selection on weapon size.

AUTHOR CONTRIBUTIONS

DJE and YH conceived of the study. JDS, YH, and GB conducted the fieldwork. RB and CEA performed the statistical analyses. DJE, JDS, and RB took the lead in writing the manuscript. All authors contributed to writing and revising the manuscript.

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DATA ARCHIVING

All data and code will be deposited in Dryad https://doi.org/10.5061/dryad.djh9w0vxw

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Scaling relationships between male horn length and body size (prothorax width) for 5 populations of *Trypoxylus dichotomus* included in this study.

Figure S2. Interaction plot of Estimated Marginal Means based on the model presented in Table 3 of the interaction between pronotum width and horn length (emmip: ‘emmeans’).

Figure S3. Interaction plot of Estimated Marginal Means based on the model presented in Table 3 of the interaction between horn length and population (function emmip in R package ‘emmeans’).

Table S1. Estimating the body size switch points between minor and major males’ allometries using the function *segmented* in the package ‘segmented’ for each population (Eberhard and Gutierrez 1991; Knell 2009).

Table S2. Slopes of the relationship between log10 (horn length) and log10(pronotum width) for major and minor males in each population. An isometric slope corresponds to a slope of 1.

Table S3. Analyses of the differences between populations in the scaling relationship between body and horn size for minor and major male. Results of a type I ANCOVA are presented here.

Table S4. Pairwise comparisons of intercepts between populations using Post hoc Tukey Honest Significant Differences (function: TukeyHSD)