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Chasin' Tail in Southern Alabama: Delineating Programmed and Stimulus-driven Grooming in Odocoileus virginianus

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Chasin' Tail in Southern Alabama: Delineating Programmed and Stimulus-driven Grooming
in *Odocoileus virginianus*

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Dedication

    This work is dedicated to the memory of my uncle, Honore Joseph Canty Sr. Our time spent hunting in Braithwaite will always be cherished, and your tenacity of life will always guide me.

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ABSTRACT

This study examined variation in ectoparasite density and grooming behavior of naturally occurring white-tailed deer (*Odocoileus virginianus*) in southwest Alabama. Stimulus-driven grooming as well as the intraspecific body size and vigilance principles of programmed grooming were tested. During the rut, males had a higher average tick (Ixodidae) density than females and exhibited complete separation of tick parasitism between non-rutting and rutting periods, supporting the vigilance principle. Stimulus-driven grooming was supported, as both fawns and yearlings had significantly higher fly (Hippoboscidae) and combined fly/tick densities than adults, and fawns oral groomed at a significantly higher rate than adults, even in the absence of allogrooming. Programmed and stimulus-driven grooming of deer examined in this study were not mutually exclusive but ectoparasite dependent.

White-tailed deer, grooming, ectoparasites, Hippoboscidae, Ixodidae, generalized linear models

INTRODUCTION

Behavioral Ecology and Dimorphism in White-tailed Deer

Understanding sexual and developmental dimorphism in the ecology of white-tailed deer is pertinent to elucidating patterns of parasitism-related behaviors. The study of different forms of grooming should take into account how behavior and morphology differ between sexes, cohorts, and seasons (intrinsic components). Ectoparasite loads are also expected to vary in space and time (extrinsic components). As grooming behavior is integral to the life history of white-tailed deer (*Odocoileus virginianus*), it can be predicted to respond to the abundance and identity of local parasites.

In Cervidae and other animals possessing male weaponry, sexual dimorphism can be observed in species where antlers are utilized in female assessment of male genetic quality, intraspecific male-male competition, dominance displays to other males, and predator defense (Clutton-Brock, 1982). Fawns (0.5 years) occasionally develop antler "buttons" several months after losing their spotted pelage, which occurs approximately four months after birth (Ditchkoff, 2011), and maximum antler growth is attained at 4.5+ years of age (Strickland & Demarais, 2000). Male and female biological roles are reflected in their morphological evolution.

Males are generally larger than females. Females reach maximum body mass from two to four years of age, and males continue growth until approximately five years of age. This is due to sex-specific reproductive strategies; females invest heavily in gestation, whereas males invest in reproductive success in the form of breeding opportunities acquired through dominance (Clutton-Brock et al., 1981; Ditchkoff, 2011). White-tailed deer not only exhibit sexual dimorphism but also are developmentally dimorphic.

Developmental dimorphism can be observed amongst fawns, yearlings (1 - 1.5 years), and adults. The majority of body mass is gained by 2.5 years of age (Leberg and Smith, 1993) and begins to level off in subsequent age classes of mature individuals. This is true for males and females, although males tend to grow at a higher rate than females during gestation and post birth, as larger males are thought to hold a higher potential of reproductive success in polygynous species (Trivers, 1972; Clutton-Brock et al., 1981). Upon birth, fawns weigh upwards of 4.1 kg (Verme, 1989), yearling males anywhere from 30 to 50 kg, and mature males 60 to 180 kg (Ditchkoff, 2011).

With regards to general behavior, most sexual differences are a result of seasonality, particularly the rut that moderates behavioral shifts between males and females in addition to yearling and prime-age bucks (Ozoga and Verme, 1985). McCullough et al. (1989) showed habitat use between males and females differs significantly during the winter and breeding seasons where males inhabit hardwood forests and females tend to exploit grasslands. Sexual segregation is pervasive in dimorphic ruminants and generally defined as "the differential use of space (and often habitat and forage) by sexes outside the mating season" (Bowyer, 2004). Male and female white-tailed deer are typically segregated outside of the rut, with males forming fraternal bachelor groups and females remaining with their matriarch doe and fawns (Hirth, 1977).

Male and female white-tailed deer show differential use of space. Home ranges vary greatly in order to acquire appropriate nutrition (Barboza and Bowyer, 2000), with males having larger home ranges than females. Male home ranges increase during the rut to increase mating probability (Nelson and Mech, 1984), and female home ranges tend to decrease during parturition. Fawns increase home range size at approximately four to eight weeks of age to

remain in close proximity of the dam and other deer (Ozoga et al., 1982). Habitat and seasons

can also influence home range, which typically decreases in size as winter severity and resources

increase (Stewart et al., 2011). Given that parasites may have microhabitat preferences,

differential use of space by deer could potentially affect the likelihood of acquiring ectoparasites.

Ectoparasites

North American white-tails are hosts to several ectoparasites, of which ticks are the most

detrimental. Members of Ixodidae (hard ticks) are known to be a primary stressor of deer by

causing anemia, weight loss, pelage loss, behavioral problems (Durden et al., 1991), and they

also transmit pathogens (Campbell and VerCauteren, 2011). According to Durden et al. (1991),

the three most common ticks in Alabama are *Ixodes scapularis* (blacklegged deer tick),

Dermacentor albipictus (winter tick), and *Amblyomma americanum* (lone star tick). Adult *I.*

scapularis are found in highest densities during the winter (in addition to more than any other

tick), but their immatures are not common on white-tailed deer. *D. albipictus* parasitizes deer

mostly during the fall, winter, and spring (Bishopp and Trembley, 1945), and *A. americanum* is

primarily a summer tick (Durden et al., 1991). Most ticks parasitize their hosts for hours or days,

but the non-host phases of their life cycle are relatively long (McCoy et al., 2013). In red deer,

tick burden was found to be sex-related and may be linked to sexual segregation, as sex and age

were significant predictors of tick density. At least in male red deer, it has been suggested that

intrinsic factors (e.g., size and age) play a greater role in predicting tick densities than extrinsic

environmental factors, with the opposite trend found in females (Ruiz-Fons et al., 2013).

In addition to ticks, Hippoboscidae (deer keds) are also considered a significant

ectoparasite of white-tailed deer due to their abundance (Demarais et al., 1987). Hippoboscids

are obligate blood-feeding ectoparasites of mammals, and the only known deer ked associated with southeastern white-tails in the United States is *Lipoptena mazamae* (Kern, 2003), possibly a vector of *Bartonella* bacteria that infect vertebrate erythrocytes (Reeves et al., 2006). Deer keds are flattened dorso-ventrally and drop their wings upon finding a suitable host (Kern, 2003). In addition to *L. mazamae* that ranges from South Carolina to Texas, three other species parasitize white-tailed deer and other cervids in North America: *Lipoptena cervi* is found on white-tails in the northeast, *Lipoptena depressa* is found on *Odocoileus hemionus columbianus* (black-tailed deer), *O. h. hemionus* (mule deer), and white-tails throughout the west, northwest, and northcentral United States and Canada, and *Neolipoptena ferrisi* commonly parasitizes *O. h. hemionus* and white-tails in the western United States (Bequaert, 1953 in Davis, 1973; Allan, 2001). In regards to management, any reduction in stressor input (ticks and flies) should prove beneficial to a population (Demarais et al., 1987).

Programmed and Stimulus-driven Grooming

Parasite defense grooming is regulated by a central control mechanism via hormonal modulation (Meisenberg, 1988) and cutaneous stimulation (Riek, 1962 in Mooring et al., 2000). Within dimorphic species of terrestrial mammals, these regulations can be explained by programmed and stimulus-driven grooming models. Programmed grooming, controlled by an ultradian clock of the central nervous system (CNS), can occur in the absence of any bite stimulus and is predicted by the intraspecific body size (Hart et al., 1992) and vigilance principles (Hart et al., 1992; Mooring and Hart, 1995). The intraspecific body size principle predicts that smaller individuals will groom more than larger individuals to prevent attachment of ectoparasites, and therefore will house fewer parasites (developmentally dimorphic

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grooming). This is due to smaller or younger individuals having a greater surface area-to-mass ratio than larger or older individuals, and thus younger individuals sustain a higher cost of infestation (Hart et al., 1992; Mooring et al., 2000). The vigilance principle states that breeding males of a polygynous species will groom less than females and bachelor males during a reproductive period (sexually dimorphic grooming). This can be due to changes in time budgets, leading to an increased level of vigilance for oestrous females and rival males, or be attributable to physiological suppression, which results from increased levels of testosterone, as demonstrated in domestic goats (Mooring et al., 1998; Kakuma et al., 2003).

In contrast, stimulus-driven grooming suggests grooming bouts will occur from parasitic irritation, and therefore predicts that animals with a greater number of parasites should groom more (Hart et al., 1992; Mooring & Samuel, 1998). Although programmed and stimulus-driven grooming are not mutually exclusive, one model may explain grooming activity more than the other (Hawlena et al., 2008). These models imply that an optimal grooming rate should balance the cost of parasitic infestation against the costs of grooming (Mooring et al., 2002).

Objective

This study seeks to test predictions of programmed and stimulus-driven grooming models in *Odocoileus virginianus*. If grooming is controlled largely by the programmed model, then juveniles (fawns/yearlings) should groom more than adults and carry fewer ectoparasites due to prophylactic grooming. Males should also carry more parasites than females during the rut from a lower rate of grooming due to changes in vigilance and/or increased testosterone. However, if the stimulus-driven model explains grooming, individuals with higher parasite loads should groom at a higher rate. This study not only seeks to assess predictions based on parasite loads of

Ixodidae, but due to the higher density of deer keds in relation to ticks per individual in the focal population, it also assesses the predictions of parasite loads by Hippoboscidae.

METHODS

Study Site

This study was conducted on a natural population of white-tailed deer in Clarke and Wilcox counties, Alabama, U.S.A. during September '13 – January '14 and September '14 – February '15. The site was owned by Soterra LLC, leased by Hardwood Hunting Club, and consisted of 5.43 km^2 of predominantly deciduous bottomland hardwoods and evergreen forest. Less common habitat included mixed forest, shrub land, grassland, pasture, wooded wetlands, and open roadway. Thirteen food plots ranging from approximately 320-9,300 m^2 were planted in April and September for the spring and winter, respectively, to supplement population nutrition. Five spring plots consisted of lablab (*Lablab purpureus*), cowpeas (*Vigna unguiculata*), and milo (*Sorghum bicolor*). All thirteen winter plots were comprised of rape (*Brassica napus*), crimson clover (*Trifolium incarnatum*), chicory (*Cichorium intybus*), wheat (*Triticum aestivum*), oat (*Avena sativa*), and rye (*Secale cereale*). Anthropogenic impact was minimal, but commercial logging was an occasional occurrence.

Quantifying Ectoparasite Load

Parasite density was quantified through the examination of males and females harvested during the 2013 (October 15 – January 31) and 2014 (October 25 – February 10) hunting seasons. Using a Petco two-sided flea comb, each individual was examined from the ventral

midline to the left lateral surface and from the chest to the anus only where white hair was present. All ectoparasites were stored in 75% alcohol and identified to species based upon general morphology (Needham, 2011). Quantities were doubled to approximate total ectoparasite load present on each deer's ventral surface, being that there was no reason to suspect one side of an individual housed more parasites than the other. Quantifying ventral parasite load and not total density per deer was sufficient since only a consistent representation of parasites was needed for analysis. Sex, age, mass, and month were also recorded at this time.

Age was estimated based upon dentition, including the presence, absence, and/or wear of milk teeth, sixth molar, cusps, dentine, and enamel (Cain and Wallace, 2003). Individuals were aged in half-year increments assuming conception in January and a seven month gestation period (Haugen, 1959). Three cohorts were established including fawns, yearlings, and adults as recommended by Gee et al. (2002). We determined mass using a Cabela's Big Buck Scale before individuals were field dressed.

Quantifying Grooming

Behavioral observations were conducted from September '13 – February '14 and September '14 – February '15 on food plots using box stands (enclosed hunting platforms), making the viewing of deer inconspicuous. All deer were observed with Nikon Action 7x35 mm binoculars and assumed to be different individuals. Continuous recording and behavior sampling (Martin and Bateson, 2007) were implemented to document strictly grooming behavior on multiple individuals simultaneously. Oral (licking and gnawing) and scratch grooming of fawns, females, and males of all ages present on food plots were recorded, including autogrooming and allogrooming. Grooming bouts lasting less than five seconds were recorded as rates (events ·

min^{-1}), whereas bouts lasting longer than five seconds were recorded and combined as total

durations (min · min^{-1}), expressed as proportions (Martin and Bateson, 2007). Individuals were

aged in half-year increments based upon morphological features including snout shape, torso

size, posture, and musculature (Meares, 2001). The month of observation was also noted.

Analytical Design

All statistical analyses were performed using R version 3.0.1 (R Core Team, 2013).

Variation in ectoparasite density was modeled using generalized linear models (GLMs) with a

negative binomial error distribution and logarithmic link function since the response variable was

discrete in the form of count data. We used a negative binomial distribution due to

overdispersion when models were fitted with a Poisson distribution (Ruiz-Fonz et al., 2013).

Predictors included mass, cohort, sex, and rut. Individuals harvested during January and

February were recorded as being 'in rut' based upon first observations of fawns and the diffusion

of bachelor groups. Mass was coded as a numeric predictor, whereas cohort, sex, and rut were

coded as factors. Fully saturated models were reduced to the most parsimonious form in a

backwards stepwise manner using the 'step' function based upon Akaike Information Criteria

(AIC; Akaike, 1974). Variance inflation factor analysis (VIF) was used to detect

multicollinearity among model predictors (Zuur et al., 2010). All predictors that yielded a VIF

value >10 were subsequently removed from the model in a backwards stepwise manner. Final

models were validated using the Pearson X^2 Goodness of Fit test. Lastly, model residuals were

extracted using the 'resid' function and tested for normality using the Shapiro-Wilk test.

Variation in grooming rates and total durations were analyzed using a non-parametric

approach due to highly skewed data with many zeros. A single yearling was observed during the

study and not included in statistical analyses. We opted to use the Mann-Whitney U test to compare mean values of independent samples (Mooring et al., 2006).

The 'MASS' library was used for modeling (Venables and Ripley, 2002) and the 'car' package for VIF analyses (Fox and Weisberg, 2011). The 'ggplot2' (Wickham, 2009) package was used for graphical development.

RESULTS

Variation in Ectoparasite Density

Average values and standard deviations of ectoparasite density per cohort and sex were calculated for each parasite family and combined as an estimate of total ectoparasite load (Table 1). *Lipoptena mazamae* (Diptera, Hippoboscidae) was the most prominent ectoparasite, found on all harvested individuals. *Dermacentor albipictus* was the most abundant tick, only found in January, followed by *Ixodes scapularis* in November and January, and *Amblyomma americanum* in January and February (Ixodida, Ixodidae).

In reference to Ixodidae, the interaction between sex and rut was removed from the final model due to quasi-complete separation. A single individual out of rut was parasitized by ticks ($N_{female} = 1/5$, $N_{male} = 0/3$), and all individuals in rut were parasitized by ticks ($N_{female} = 2/2$, $N_{male} = 7/7$; Figure 1). Males not only exhibited complete separation of tick parasitism at the

Table 1 | Average ectoparasite density (parasites / deer) and standard deviation per cohort and sex with respect to Ixodidae (Ixo), Hippoboscidae (Hip), and total ectoparasite load.

Sex	Cohort	N	Ixo	Hip	Total
Female	Fawn	0	NA	NA	NA
	Yearling	2	3.0 ± 4.2	34.0 ± 22.6	37.0 ± 18.4
	Adult	5	3.2 ± 4.6	55.2 ± 32.2	58.4 ± 30.8
Subtotal Female		7	3.1 ± 4.1	49.1 ± 29.7	52.3 ± 28.2
Male	Fawn	2	8.0 ± 5.7	46.0 ± 62.2	54.0 ± 56.6
	Yearling	2	0.0 ± 0.0	88.0 ± 87.7	88.0 ± 87.7
	Adult	6	19.0 ± 20.6	35.7 ± 24.0	54.7 ± 34.9
Subtotal Male		10	13.0 ± 17.5	48.2 ± 45.4	61.2 ± 45.7
Total		17	8.9 ± 14.3	48.6 ± 38.6	57.5 ± 38.6

NA, not applicable

Figure 1 | Boxplots and mean values (red dots) of interactive effect between sex and rutting activity on ectoparasite density (ticks / deer).
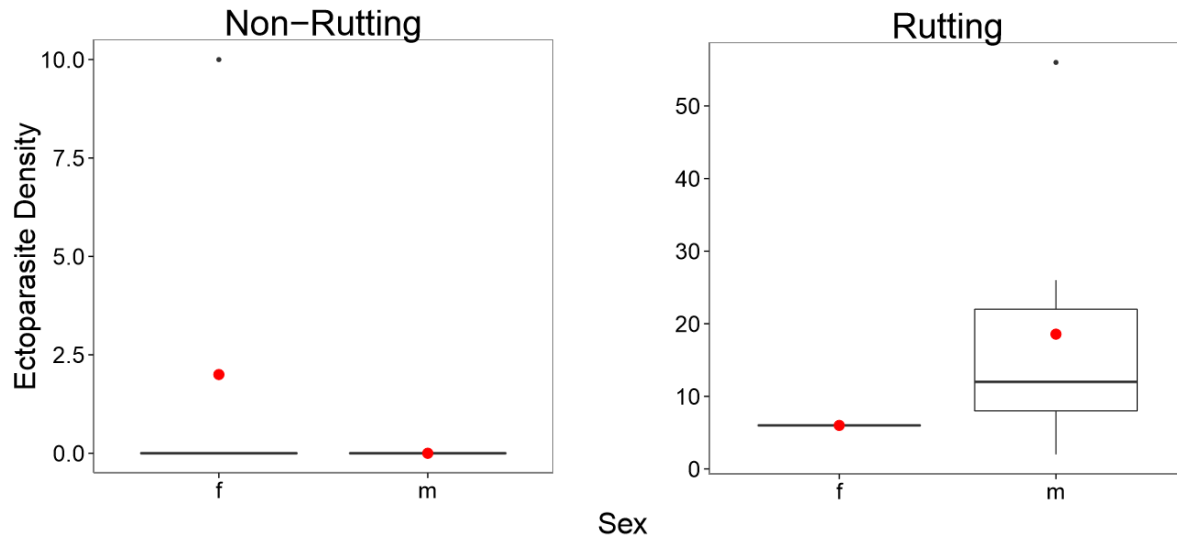
onset of rutting activity but, on average, had a higher tick density than females in rut. After removing the partial separation from the model, variation in tick density was explained most parsimoniously by sex; males had significantly more ticks than females (Table 2). Mass, cohort, and rut were not significant predictors of tick density.

The interactions between mass and cohort, as well as mass and rut, best explained variation in fly density. Hippoboscid density decreased significantly with age (per cohort) for every one unit increase in mass. Individuals in rut had more flies per unit mass than individuals out of rut (Table 2). The individual predictors mass, cohort (Fig 2), and rut (Fig 3) were therefore retained in the final model, allowing significant interactions with covariates to be considered. Sex was not a significant predictor of hippoboscid density.

Total ectoparasite load followed a similar pattern to what was found for Hippoboscidae alone. When combined, tick and fly density decreased significantly per cohort as mass

increased. When considering mass, total ectoparasite load was higher on individuals in rut than

out of rut (Table 2). Single predictors were significant and retained in the final model

(Figs 2, 3). Sex was not a significant predictor of total ectoparasite load.

Table 2 | Statistical results (coefficient / z-value and significance level: − not retained, #0.1, *0.05, **0.01, ***0.001) of generalized linear models (negative binomial error distribution and logarithmic link function) created to predict ectoparasite density per deer with respect to Ixodidae (Ixo), Hippoboscidae (Hip), and total ectoparasite load.

Predictor	Ixo	Hip	Total
Mass	−	-0.0569 / -2.719 **	-0.0517 / -2.558 *
Fawn [a]	−	-12.4638 / -3.838 ***	-4.9464 / -2.247 *
Yearling [a]	−	-5.3032 / -2.477 *	-5.0875 / -2.452 *
Sex [b]	1.4200 / 1.647 #	−	−
Rut [c]	−	-3.3689 / -2.735 **	-3.8413 / -3.237 **
Mass:Fawn [a]	−	0.4206 / 4.055 ***	0.1913 / 2.659 **
Mass:Yearling [a]	−	0.1241 / 2.420 *	0.1205 / 2.424 *
Mass:Rut [c]	−	0.0592 / 2.536 *	0.0720 / 3.201 **
Intercept	1.1451 / 1.705 #	6.7977 / 6.502 ***	6.5604 / 6.488 ***

[a] in relation to adults
[b] males in relation to females
[c] in rut as related to out of rut

Figure 2 | Boxplots and mean values (red dots) of ectoparasite density (parasites / deer) per cohort with respect to Hippoboscidae and total ectoparasite density.
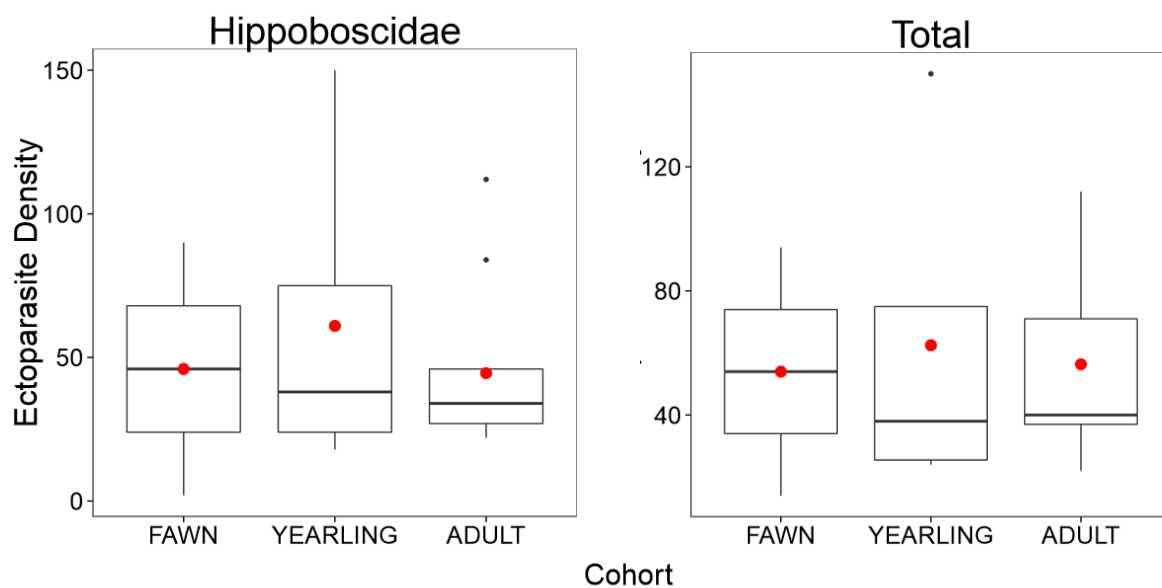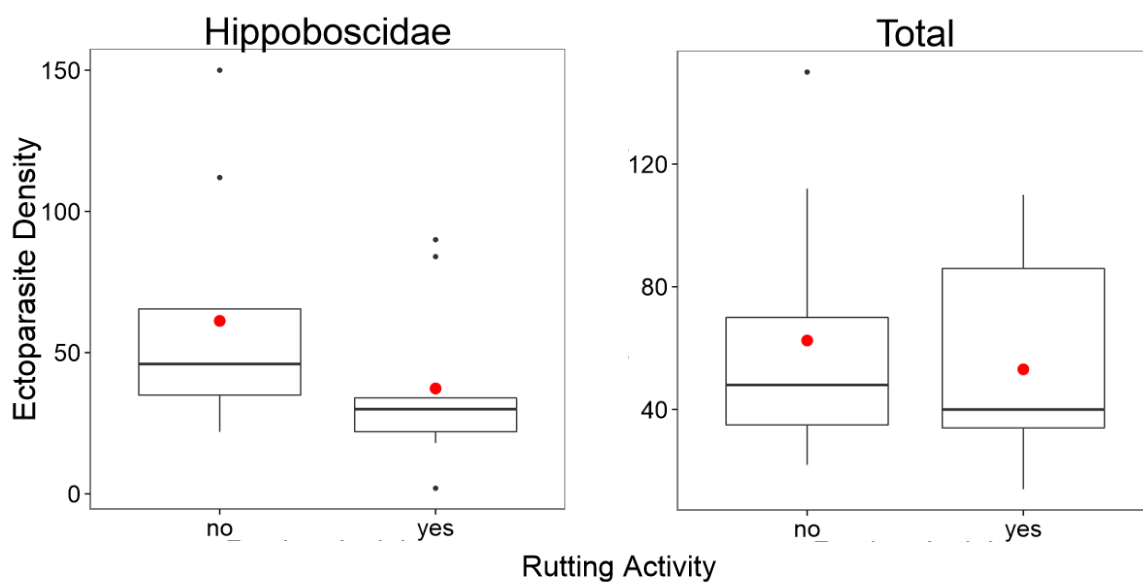

Figure 3 | Boxplots and mean values (red dots) of ectoparasite density (parasites / deer) of deer in and out of rut with respect to Hippoboscidae and total ectoparasite density.

Variation in Grooming Behavior

The cautious demeanor of deer creates challenges in collecting adequate data from a wild population (Haugen, 1959). Individual deer were observed for approximately 16 of the 150 hours of field work. Averages and standard deviations per cohort and sex were quantified for each type of grooming behavior. Not all observed fawns were sexed during this study (Table 3).

Table 3 | Averages and standard deviations of grooming rates (events · min^{-1}) and total durations (min · min^{-1}) of combined autogrooming and allogrooming (OR oral rate, SR scratch rate, OD oral duration) per cohort and sex.

Sex	Cohort	N	OR	SR	OD
Female	Fawn	0	NA	NA	NA
	Yearling	0	NA	NA	NA
	Adult	15	0.0100 ± 0.0236	0.0041 ± 0.0096	0.0010 ± 0.0032
Subtotal Female		15	0.0100 ± 0.0236	0.0041 ± 0.0096	0.0010 ± 0.0032
Male	Fawn	2	0.0423 ± 0.0598	0	0
	Yearling	1	0	0	0
	Adult	4	0.0211 ± 0.0423	0	0
Subtotal Male		7	0.0241 ± 0.0412	0	0
	Fawn [a]	14	0.0426 ± 0.0446	0.0159 ± 0.0279	0.0018 ± 0.0031
Total		36	0.0254 ± 0.0384	0.0079 ± 0.0192	0.0011 ± 0.0028

[a] sex not determined
NA, not applicable

Combined autogrooming and allogrooming oral rates of fawns were significantly greater than adults (Mann-Whitney U test: W = 90, N_{fawns} = 16, N_{adults} = 19, p < 0.05; Fig 4). Oral

grooming rates did not differ between females and males (W = 50, $N_{females}$ = 15, N_{males} = 6, p = 0.6604) nor rutting and non-rutting periods (W = 90, N_{rut} = 8, $N_{non-rut}$ = 27, p = 0.4459).

Scratch grooming rates did not differ between fawns and adults (W = 181, N_{fawns} = 16, N_{adults} = 19, p = 0.1995). No males scratch groomed (N = 0/6), but some females did (N = 3/15). No scratch grooming occured during the rut (N = 0/8), and no scratch grooming durations were observed during this study (N = 0/36).

Oral grooming durations did not differ between fawns and adults (W = 174, N_{fawns} = 16, N_{adults} = 19, p = 0.2784). No males oral groomed in the form of durations (N = 0/6), but females did (N = 2/15). No oral grooming in the form of durations was observed during the rut (N = 0/8). When allogrooming was excluded from modeling, autogrooming oral rates and durations were analyzed independently.

Fawn oral autogrooming rates were significantly greater than adults (W = 218, N_{fawns} = 16, N_{adults} = 19, p < 0.05; Fig 4). Oral autogrooming rates did not differ between males and females (W = 38, $N_{females}$ = 15, N_{males} = 6, p = 0.4981), and autogrooming rates did not differ between rutting and non-rutting periods (W = 93, N_{rut} = 8, $N_{non-rut}$ = 27, p = 0.52).

Oral autogrooming durations did not differ between fawns and adults (W = 164, N_{fawns} = 16, N_{adults} = 19, p = 0.5138). No males oral autogroomed in the form of durations (N = 0/6), but females did (N = 2/15). No oral autogrooming durations were observed during the rut (N = 0/8).

Figure 4 | Boxplots and mean values (red dots) of total oral and oral autogrooming (allogrooming removed) rates between fawns and adults.
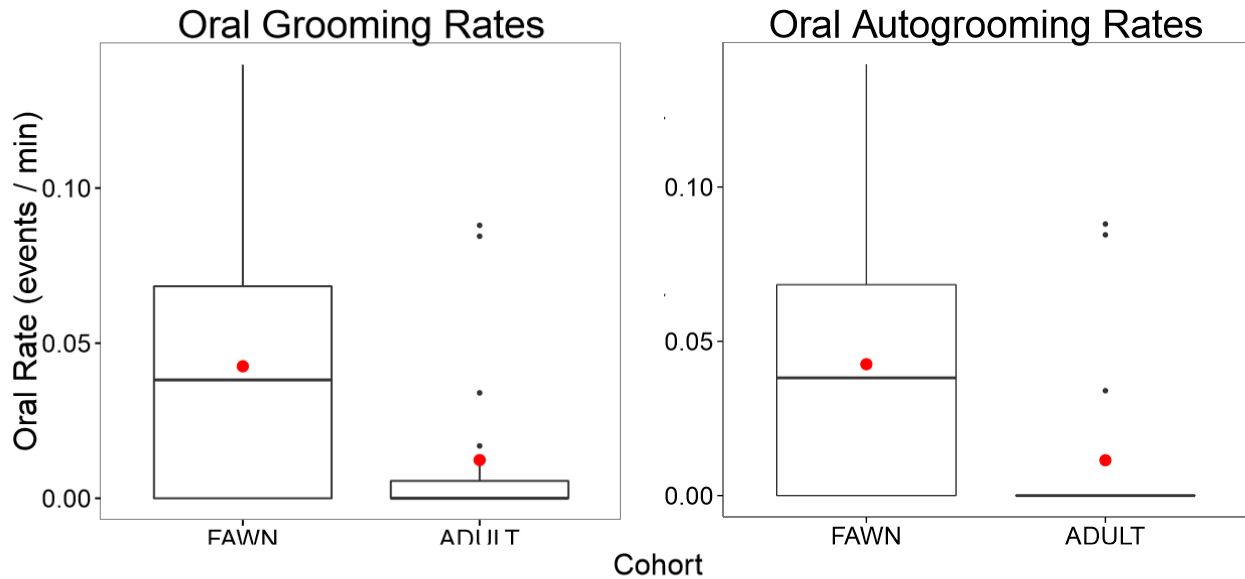
DISCUSSION

Body Size and Vigilance Principles

This study provided support for the stimulus-driven grooming model with respect to *L. mazamae* (Hippoboscidae) and total ectoparasite load. However, it did not support the intraspecific body size principle of programmed grooming in white-tailed deer for Ixodidae, Hippoboscidae, or total ectoparasite load. Juveniles (fawns and yearlings) had significantly higher fly and combined fly/tick densities than adults per unit increase in body mass (Table 2), but these densities alone do not indicate that individuals groomed responsively to external stimuli. In wild populations, we must also consider grooming behavior to determine if parasitic trends are a result of proximate agitation or an evolved programmed response. Significant trends

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in parasitism, combined with significantly higher oral grooming rates in fawns versus adults do provide support for responsive grooming. Higher densities of hippoboscids on both fawns and yearlings (compared to adults) were associated with significantly higher rates of oral (combined auto/allogrooming) and oral autogrooming for fawns versus adults (Figure 4). This trend also holds for total ectoparasite load, considering both flies and ticks as detrimental ectoparasites.

The average values of ectoparasite densities for fawns and yearlings indicate that yearlings had a greater hippoboscid density than fawns, and adult total ectoparasite load was greater than fawns (Figure 2). We suspect that this occurrence may be due to the small sample size of harvested fawns (Table 1), as fawn and young buck recruitment are encouraged on managed property to produce desirable mature bucks (Jacobson et al., 2011). The predominant support for stimulus-driven grooming lies in the differences between fawns and adults, in that we were unable to observe enough yearling grooming behavior for statistical analysis. As mass increases with age, the interaction between these two variables best explains the decrease in ectoparasite load.

This study found significantly higher tick densities on males than females (Table 2). However, due to quasi-complete separation for the interaction between sex and rutting activity not included in modeling (Fig 1, Table 2), it is likely that distractions from vigilance played a role in mediating the increase in male tick density during the rut, thus supporting the vigilance principle of programmed grooming for Ixodidae. Although the primary focus of the vigilance principle is male and female ectoparasite densities and grooming rates during the rut, it seems evident that these parameter values change between rutting and non-rutting periods as well.

Complete separation of male tick densities in and out of rut parallels the hypothesis that male ectoparasite density should be greater than females and sexually immature males during the

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rut. This reflects temporal changes as males begin to exhibit rutting behavior (e.g., chasing, tending, mating, and fighting rivals). Indeed, this study found that ticks parasitized males more than females during the rut (Figure 1). *Post hoc* analyses were not employed to analyze tick variation for the subset of males and females during the rut, but the separation and higher rate of parasitism on males during the rut supports the vigilance principle. Future field studies should seek a higher sample size which would be particularly important if *post hoc* analyses are deemed necessary in light of partial separation.

Finally, we also note that variation in tick parasitism regarding rutting behavior should not be attributed to fluctuations in environmental tick abundance, as the density of all three identified tick species vary differently during a given year (Durden et al., 1991). With that said, grooming rates are predicted to adjust accordingly on a seasonal basis, as tick (ectoparasite) challenge fluctuates temporally (Mooring et al., 2004).

Grooming rates and total durations between males and females during the rut could not be analyzed, as only a single female was observed during the rut. One fawn and one adult were observed concurrently of five males during the rut, and both groomed at an oral rate of 0.0845 events $\cdot$ min $^{-1}$. The single adult female observed during the rut did not groom.

Programmed grooming may not only be due to distractions from vigilance during the rut but also physiological suppression by testosterone (Mooring et al., 1998; Kakuma et al., 2003). Testosterone levels of harvested males were not quantified for this study. Nevertheless, as testosterone levels increase during periods of mating (Bubenik et al., 1990), the associated peak rutting activity (January and February) in the focal population was utilized as a model factor rather than direct measures of testosterone.

Overall, grooming in white-tailed deer of southwest Alabama was predominately stimulus-driven with an underlying rate of programmed grooming. Programmed grooming for ticks should be considered within an evolutionary framework, as selective pressures may have previously shaped current behavioral aspects of cervids during the evolution of artiodactyls (Mooring et al., 2002). Conversely, stimulus-driven grooming in response to flies can be considered as a proximate response to agitation. This study supports the concept that these models of grooming are not mutually exclusive (Hawlena et al., 2008) but parasite and host dependent.

Applications

Our results increase basic knowledge of white-tailed deer behavioral ecology and have practical applications for parasite control and deer management. Given that tick and fly densities fluctuate significantly in space and time, the control of ectoparasite loads can be incorporated into deer management. Current tactics used to control the spread of ectoparasites include herbicide treatment using 4-poster bait stations (Carroll et al., 2009) and habitat manipulation, including controlled burning (Jacobson and Hurst, 1979) and the disking of food plots (Jacobson et al., 2011). Our results provide additional information that can supplement the location and timing of these approaches.

Knowing that ticks are more prevalent on males than females, primarily during the rut, the placement of 4-posters may prove most beneficial where bucks are found at particular times of the year. This should be determined on a population basis, as land cover and breeding periods vary across white-tailed deer geographical range. During parturition, control measures should also be established near the bedding areas of fawns, where hippoboscids tend to pupate

(Allan, 2001; Kern, 2003). Reducing fly densities where juveniles are most affected could potentially reduce initial densities per fawn/yearling and the horizontal transmission to other individuals. If initial fly parasitism is reduced, a decrease in stimulus-driven grooming should therefore follow, resulting in reduced stress and/or an increase in energy conservation within the population.

Direct manipulation of land cover can also help ectoparasite control and be beneficial to deer management (e.g., population density, sex ratio, etc.). Habitat manipulation can allow deer to circumvent certain land cover types that harbor detrimental ectoparasites. For example, increased sunlight by clear-cutting and/or thinning dense habitat and food plots could promote tick desiccation (Jacobson et al., 2011).

Ectoparasite densities with respect to land cover have also been considered within the programmed grooming model: the habitat (Hart et al., 1992) and tick challenge principles (Mooring, 1995 in Mooring et al., 2004). These concepts were not considered in our study due to habitat heterogeneity and a need to quantify the spatial use of landscapes by deer (see below).

Implications for Future Research

Within the programmed grooming model, four principles predict a baseline rate of grooming in the absence of external stimuli. Our study supported the vigilance principle and refuted the intraspecific body size principal of programmed grooming. However, the habitat and tick challenge principles remain untested in natural populations of white-tailed deer because such tests would require a more exhaustive approach than what was possible in this study.

To address the habitat and tick challenge principles, it would be necessary to chart environmental characteristics (e.g., land cover, sunlight exposure). However, quantifying

percent land cover within each deer's estimated home range does not equate to the time spent by each individual within each type of land cover (Figure 5; Esri; Jin et al., 2013). For example, an individual's home range may include 50% deciduous forest, but one should not assume that it spends 50% of its time there. Due to the heterogeneous habitat in southwest Alabama, testing the habitat and tick challenge principles would require recording time budgets of each deer regarding land cover. Environmental ectoparasite density (tick challenge) and habitat type (habitat principle) could then be correlated with grooming behavior while considering the movement of deer within a heterogeneous environment. Habitat has been correlated to the grooming behavior of cervids and bovids, but habitat types were ranked according to literary support (i.e., scaled 1-6 based upon habitat density; Mooring et al., 2000; Mooring et al., 2004). For future studies, we recommend that a direct approach be taken for white-tailed deer, as opposed to assuming homogeneous land cover.

Figure 5 | Average ectoparasite load per deer per harvest location with respect to land cover. Pie chart diameter also represents average home ranges of resident Alabama white-tailed deer (81 ha; Marchington and Hirth, 1984 in Stewart et al., 2011).
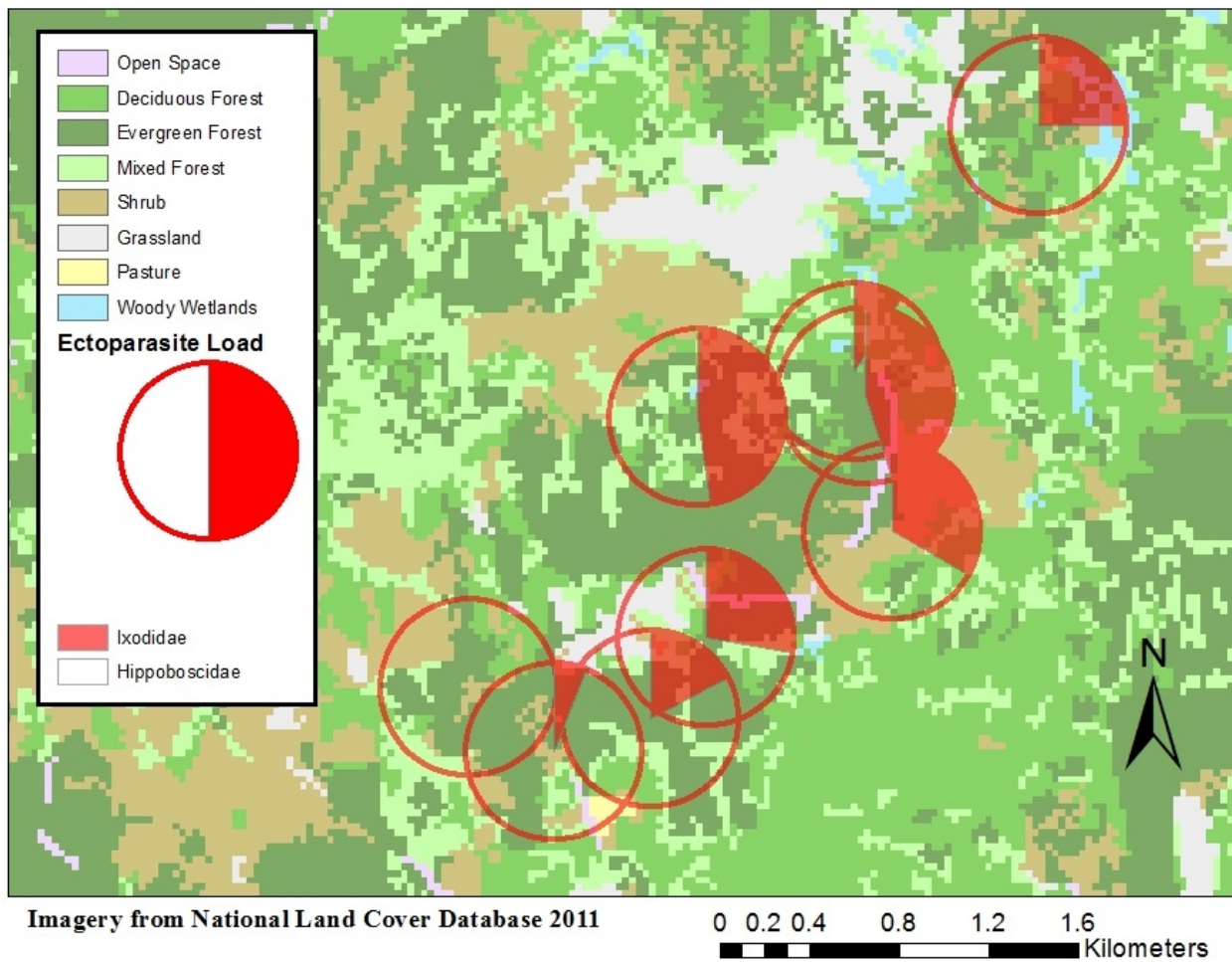
To our knowledge, this is the first study to test programmed and stimulus-driven grooming in wild white-tailed deer that includes other ectoparasites (flies) in addition to ticks. Studies of grooming in captive dimorphic terrestrial mammals provided information about the evolution of grooming behavior by controlling for stimulus-driven grooming (e.g., Mooring et al., 2000; Mooring et al., 2002; Mooring et al., 2004). However, a multivariate approach to

studying parasitism as it relates to grooming in wild populations is yet to be explored; this will

provide us with a more precise view into the natural history of these species and also a broader

approach towards the evolution of grooming. Our hope is that this work contributes to

knowledge of grooming and parasitism in one of North America's most common game species

and inspires future work in white-tailed deer behavioral ecology.

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