

A comparative study of aggressiveness in eastern North American field cricket species (genus *Gryllus*)

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Received: 6 July 2007 / Revised: 14 January 2008 / Accepted: 20 February 2008 / Published online: 29 March 2008
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Abstract To understand the variation in aggressiveness and factors important for contest outcome, we quantified and compared agonistic interactions of four field cricket species in eastern North America: *Gryllus fultoni* (Orthoptera; Gryllidae), *G. vernalis*, *G. pennsylvanicus*, and *G. rubens*. The most aggressive behavior that we observed, the grapple, was frequently displayed in agonistic trials of *G. pennsylvanicus* and *G. rubens*. By contrast, we never observed this behavior in trials involving *G. fultoni* and *G. vernalis*. Consequently, species was the only significant variable affecting the variation in aggressiveness, whereas size difference, age difference, and contest duration had no significant effect. In species with high levels of aggressiveness, *G. pennsylvanicus* and *G. rubens*, the factors that might be indicative of resource holding potential such as size or age difference seemed to be significant for contest outcome. In species with low levels of aggressiveness, *G. fultoni* and *G. vernalis*, however, there were indications that contest outcomes were determined by aggressiveness itself rather than the size and age differences between opponents. Markov chain analyses revealed that the difference in aggressiveness between species with high and low levels of aggressiveness lay in a sequence of escalating behaviors: antennal fencing, mandible flare, and grapple. The escalated state of aggressiveness char-

acterized by this behavioral sequence in *G. pennsylvanicus* and *G. rubens* seems to be the ancestral state in the North American *Gryllus* phylogeny. We argue that the loss of a tendency to use burrows in *G. fultoni* and *G. vernalis* might be related to low levels of aggressiveness in these species.

Keywords Aggressiveness · *Gryllus* · Resource holding potential · Resource value · Territoriality

Introduction

Why do animals differ in aggressiveness and what features are good indicators of fighting ability? Since the Tang Dynasty (618–906 AD) in China, cricket breeders have been striving to identify fighting crickets with winning combinations of morphological and behavioral traits (Jin and Yen 1998). More recently, such questions have attracted the interest of evolutionary biologists as well. Modern theoretical and experimental studies of agonistic interactions reveal that variation in animal aggression is generally a function of resource holding potential (RHP) and resource value (Parker 1974). RHP is a combination of characteristics such as body size, strength, weapons, etc. (Parker 1974). Animals with higher RHPs may escalate their aggression more as they have less to fear in a physical fight (Hurd 2006). Theories of resource value assert that individuals exhibit variation in aggression because the value of winning the contested resource differs between individuals (Maynard Smith 1982; Riechert 1998). For example, when individuals are deprived of food for an extended length of time, they become more aggressive and often win contests, even against larger opponents (see Hack 1997; Bradbury and Vehrencamp 1998; Johnsson and Forser 2002). Recently, Hurd (2006) defined aggressiveness as

Communicated by D. Gwynne

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the tendency to escalate a contest. Aggressiveness may in some cases be independent of RHP and resource value (Barlow et al. 1986; Maynard Smith and Harper 1988).

The individual-level approach to animal aggression has shortcomings in addressing many important questions about animal contests, such as why closely related species differ in aggressiveness and what changes in morphology or behavior are associated with changing levels of aggressiveness. Such questions are best addressed by a comparative approach in which behaviors and morphology of interest are compared among a group of related taxa (Foster and Endler 1999; Brooks and McLennan 2002). When comparative studies of traits are mapped onto phylogenetic frameworks, valuable insights concerning the evolution of the traits often emerge (Moretz and Morris 2003; for review, see Avise 2006). By teasing apart the ancestral (plesiomorphic) states of traits from the derived (synapomorphic) states, we can understand patterns of evolution of aggressiveness among closely related species and the association between adaptation and environment (Avise 2006).

We studied male intraspecific agonistic interactions in four field cricket species in eastern North America: *Gryllus fultoni* (Alexander) (Orthoptera; Gryllidae), *G. vernalis* Blatchley, *G. pennsylvanicus* Burmeister, and *G. rubens* Scudder. The morphology, life cycles, and taxonomic relationships of these four cricket species were described by Alexander (1957, 1968). The life cycles of *G. fultoni* and *G. vernalis* are characterized by one generation per year with juvenile diapause during winter (Jang and Gerhardt 2006a, 2006b, 2007). The adult stage of these two species occurs in the spring. *G. pennsylvanicus* also has one generation each year. But the eggs of this species diapause in winter, and adults of *G. pennsylvanicus* occur in the fall. Unlike the three species above, *G. rubens* has two discrete generations per year with juvenile diapause. Adults of *G. rubens* occur in early spring and in the fall. All four species are generally nocturnal. Populations of adult *G. fultoni*, *G. vernalis*, and *G. rubens* overlap spatially and temporally in the spring, and *G. rubens* also overlaps spatially and temporally with the egg-diapausing *G. pennsylvanicus* in the fall. Phylogenetic analyses based on DNA sequences suggest that *Gryllus* species in eastern North America may be divided into four clades that are characterized by different life history traits (Huang et al. 2000). In the resulting phylogenetic tree, *G. fultoni*, *G. pennsylvanicus*, and *G. rubens* occur in separate clades. Unfortunately, *G. vernalis* was not incorporated into this phylogeny. A recent preliminary DNA analysis shows that *G. fultoni*, *G. vernalis*, *G. integer*, and *G. veletis* form a clade, but that *G. fultoni* and *G. vernalis* are not sister taxa (Fig. 2; Gray, personal communication; Jang et al. 2007).

In this study, we quantified the aggressive behaviors of four cricket species and addressed the variation in aggres-

siveness and contest outcome by considering the effects of factors such as species, size difference, age difference, and contest duration. We then identified the sets of behaviors that differentiated the behavior patterns of the four cricket species. Differences in agonistic behaviors among cricket species are discussed in terms of the evolution of aggressiveness in crickets.

Materials and methods

Study species

Adult females of the four field cricket species were captured in the field, and the progeny of these crickets were reared from eggs to adults in the laboratory. We collected *G. fultoni* in May 2002 in Dawson Springs, KY (27) and Jackson, GA (35), USA. The number in parentheses represents the number of field-caught females used for egg collection. *G. vernalis* was collected in May 2002 and 2003 in Dawson Springs, KY (16) and Anderson, IN (24), USA. We collected *G. rubens* in April 2004 in Goreville, IL (34), USA. *G. pennsylvanicus* was collected in August and September 2003 in Columbia, MO (48), USA. The progeny of these crickets were reared without diapause in plastic bins (33×50×29 cm) with holes on the side for ventilation. Both juvenile and adult crickets were provided with cricket chow (Fluker Farms, LA, USA), lettuce, and shelter ad libitum. Newly emerged adults were removed within 48 h of the final molt from the stock population and were housed in individual containers (12×12×9 cm) to ensure that all crickets used for this study were virgin (Solymar and Cade 1990). We randomly selected intact males from these virgin males for trials. All trials were conducted on the first-generation crickets from the field-caught females. All crickets were maintained at 23±1°C and with a 14:10-h light/dark photoperiod during development. The age of the crickets was calculated as the number of days from the final molt to the trial.

Because *G. pennsylvanicus* is difficult to distinguish from *G. veletis* based on morphology, we used the hatching date to confirm that our samples were indeed *G. pennsylvanicus*. Field-caught females of *G. pennsylvanicus* were placed on wet sand without males in individual containers (12×12×9 cm) to lay eggs, and we recorded the number of days until the first instar hatched for each female. Forty-eight out of 55 individual containers produced instars, and the average day to hatching for the fastest-hatched instars was 76.15±15.73 days (mean±SD; range 53–130 days). At 26.7–29.4°C, nondiapause *G. veletis* eggs usually hatch within 21 days, whereas the eggs of *G. pennsylvanicus* rarely hatch in <60 days (Alexander and Bigelow 1960).

Agonistic trials

Agonistic trials were conducted in a temperature-controlled anechoic chamber (3×3×2 m). Plastic containers (12×12×9 cm) with sand bottoms were used as arenas for the trials. To standardize aggressiveness and motivation of test crickets, we used each cricket only once for agonistic trials, introduced them to test arenas that were unfamiliar to both contestants, and tested them before they established territoriality. Two randomly selected males of the same species and population were placed in the arena separated by an opaque plastic divider at least 30 min but no more than 2 h before observation. We never observed an attempt to dig in the sand during acclimation in any of the cricket pairs. The divider was then lifted and the trial was conducted for 10 min. All trials were conducted under complete darkness during the dark photoperiod. The trials were videotaped using a digital camera recorder with the infrared setting (Sony; model #: DCR-TRV30; Tokyo, Japan). The camcorder was located 1 m directly above the container. A Sennheiser microphone (ME 66 shotgun head+K6 powering module, frequency response=50–20,000 Hz±2.5 dB) was placed with its tip 75 cm above the container at an angle of about 20° with the camera recorder. Output from the microphone was fed into the Sony recorder, which was operated with a sampling rate of 48 kHz and 16 bits during trials. A thermocouple probe (Omega Engineering, model 450-AKT) placed near the crickets was used to note the exact temperature. The temperature of the anechoic chamber was maintained at 23±1°C. The ages of the crickets were 19.94±8.085 days (mean±SD; range 8–35 days) for *G. fultoni*, 14.62±2.540 days (range 8–20 days) for *G. vernalis*, 22.55±7.708 days (range 8–35 days) for *G. pennsylvanicus*, and 17.30±6.846 days (range 6–35 days) for *G. rubens*. In *G. bimaculatus*, males aged

<10 days were less likely to escalate for fighting (see Adamo and Hoy 1995). The percentages of males aged <10 days were 6.5% for *G. fultoni*, 1.2% for *G. vernalis*, 3.0% for *G. pennsylvanicus*, and 7.7% for *G. rubens*.

Agonistic interactions between male crickets involve a stereotyped sequence of escalating aggressive behaviors (Alexander 1961; Adamo and Hoy 1995; Tachon et al. 1999; Hofmann and Schildberger 2001). In Mediterranean field crickets, *Gryllus bimaculatus*, an agonistic encounter starts with antennal contact between males. Cricket antennae are critical for recognizing the opposing individual both in the mating (Adamo and Hoy 1994) and agonistic (Adamo and Hoy 1995) contexts. Antennectomized males failed to direct aggressive behaviors toward other males (Murakami and Itoh 2003). After antennal contact, two opposing males may engage in a series of escalating aggressive behaviors. See Table 1 for the definitions of behaviors used in cricket agonistic encounters and their intensity scores. A typical behavioral sequence starts with antennal fencing, and proceeds to mandible flare, and then grapple (Adamo and Hoy 1995). Hofmann and Schildberger (2001) suggest that antennal fencing is used to assess the fighting readiness of the opponent and that mandible flare may be a signal of fighting ability. Grappling, which incurs the greatest energetic cost among *Acheta domesticus* aggressive behaviors (Hack 1997), often involves interlocking of the mandibles of the opponents. Encounters may be terminated at any point within this sequence (Adamo and Hoy 1995).

Because the dynamics of agonistic interactions are fixed after the establishment of a stable dominance relationship (Khazraie and Campan 1999), we only included the agonistic interactions between two conspecific males up until the determination of the contest outcome in a 10-min trial for our behavioral analysis. Because males of *G. bimaculatus*

Table 1 Agonistic behaviors used in the ethogram (Adamo and Hoy 1995)

Behavior	Description	Intensity score
Pause (P)	Immobile for more than 15 s	0
Withdraw (W)	Moves away from tactile contact	0
Antennal fencing (AF)	Crickets rapidly antennate the other's antennae	1
Rock body (RB)	Stationary cricket rocks body rapidly back and forth	1
Kick (K)	Kicks leg toward conspecific	2
Threat posture (TP)	Raises itself on its forelegs	2
Mandible flare (MF)	Cricket hyperextends mandibles	3
Aggressive song (AS)	Stridulates aggressive song	3
Chase (C)	Runs after opponent	3
Bite (B)	Pinches opponent with mandibles	3
Lunge (L)	Rushes opponent with open mandibles	3
Grapple (G)	Crickets butt heads and/or interlock mandibles and push or pull their opponent	4
Male flipped (F)	Male flipped on its back during a grapple	No score
Antennal contact (AC)	Cricket contacts at least one antenna of its opponent with at least one antenna	No score
Body contact (BC)	Cricket contacts the body of its opponent with at least one antenna	No score

discriminate among conspecifics using cuticular pheromones on the body surface (Tregenza and Wedell 1997; Nagamoto et al. 2005) and because we observed that males initiated aggressive behaviors after body contact, we used body contact or antennal contact to mark the beginning of the contest. The outcome of a contest was determined when one individual, designated as the loser, withdrew for at least 5 s, while the other, designated as the winner, exhibited some form of aggressive behavior during this period. Previous definitions of contest outcomes focused on retreat regardless of winners' behaviors (see Adamo and Hoy 1995; Nelson and Nolen 1997). Our definition was necessary to compare contest outcomes of all four species. In some agonistic trials of *G. fultoni* and *G. vernalis*, there were no obvious or effective aggressive behaviors or two crickets dueled physically or acoustically to the end of the 10-min trial. A tie was scored in these cases. We recorded all behaviors until a loser withdrew twice in a row while the winner displayed aggressive behaviors. If these conditions were satisfied, the dominance relationship was not changed throughout the 10-min agonistic trial. All statistical analyses of outcomes and temporal structures of contests were based on behavioral interactions up to the first withdrawal.

After a trial, each cricket was placed in the solution of 75% alcohols, and the right hind leg was removed from the thorax and placed on its side. The hind femur length was measured from the base of the femur to the joint with the tibia with a venire caliper. Hind femur length is often used as a measure of overall size in orthopteran insects (Simmons 1988), but whether it correlates with other measurements of body size in the cricket species in our study is not known.

Statistical analyses

To analyze the variation in aggressiveness, each trial was given an aggressiveness score based on the aggressive behavior with the highest intensity score (see Table 1) performed by either participant in the trial regardless of the outcome of the trial. *G. fultoni* and *G. vernalis* never exhibited grapple (aggressiveness=4), and trials involving *G. pennsylvanicus* and *G. rubens* were never classified in the less-aggressive categories (aggressiveness=0, 1, 2). Because the numbers of trials scored in the three least-aggressive categories 0 ($n=9$), 1 ($n=0$), and 2 ($n=4$) were very low, we combined these three categories into one. This reduction of categories in the predictor variable left us with only one independent variable besides the intercept in any modeling. Because of the quasicomplete separation in the data, the relationship between the variation in aggressiveness and species was analyzed using the nonparametric Kruskal–Wallis test. Species included *G. pennsylvanicus*, *G. rubens*, *G. fultoni*, and *G. vernalis*. We used bivariate analyses using ordinal regression to understand the relation-

ships between aggressiveness and other predictor variables including “size difference,” “contest duration,” and “age difference.” Size difference was the hind femur length of a focal male in a contest minus that of his opponent. Age difference was the age of a focal male in a contest minus that of his opponent. We used size difference and age difference, instead of size and age, to reflect the fact that the outcome for a given individual may be influenced by the other individual in a contest (see Jang and Greenfield 1998). Contest duration was the time between the first contact and withdrawal in a trial. Contest duration for a trial with a tie outcome was designated as 600 s.

To determine factors important for the outcomes of the contest, we applied binary logistic regression analysis (1) for each species separately and (2) for datasets pooled according to the level of aggressiveness. The predictor variables evaluated were “aggressiveness,” “contest duration,” “size difference,” and “age difference.” There were no population differences in aggressiveness in the ordinal regression (Wald=0.298, $df=1$, $P=0.585$) or in contest outcome in the binary logistic regression (Wald=0.001, $df=1$, $P=0.977$). The analyses of the nonparametric, ordinal, or binary logistic regressions presented in the “Results” section were based on pooled populations for each species.

We also conducted first-order Markov chain analyses (Gottman and Roy 1990) to test for the existence of nonrandom temporal associations between behavior patterns in agonistic interactions. Of the 15 behaviors listed in Table 1, we excluded threat posture as there were no displays of this type in any of the four species, and the behaviors bite and lunge were grouped together into one category. We then constructed a 13×13 transition matrix for winners and losers of each species by tabulating all instances in which one behavioral pattern led to another (see Chen et al. 2002). We computed an expected frequency for a particular chain by dividing the total transitions by 169, the total number of cells, and then we used a chi-square goodness-of-fit test to determine the significance of the chain (Bakeman and Gottman 1997).

Results

Aggressiveness

In *G. pennsylvanicus* ($n=33$ trials) and *G. rubens* ($n=26$ trials), all agonistic trials had a clear winner and loser. However, there were no clear outcomes in 19.4% and 20.9% of agonistic trials in *G. fultoni* ($n=31$ trials) and *G. vernalis* ($n=43$ trials), respectively (Table 2). Aggressiveness was significantly different among species (Kruskal–Wallis test, $\chi^2=72.732$, $df=3$, $P<0.001$, $n=133$). In *G. pennsylvanicus* and *G. rubens*, 83% and 62% of the

Table 2 Aggressiveness scores of the four cricket species

Species		Aggressiveness				
		0	1	2	3	4
<i>G. fultoni</i> (n=31 trials)	Winner	0	1	1	22	0
	Tie	3	0	6	5	0
	Loser	11	0	6	7	0
<i>G. vernalis</i> (n=43 trials)	Winner	0	0	2	32	0
	Tie	10	0	2	6	0
	Loser	26	0	3	5	0
<i>G. pennsylvanicus</i> (n=33 trials)	Winner	0	0	0	8	25
	Loser	3	1	1	3	25
<i>G. rubens</i> (n=26 trials)	Winner	0	0	0	10	16
	Loser	5	2	0	3	16

Aggressiveness was the aggressive behavior with the highest level of the intensity score in a trial (see Table 1). Agonistic interactions of *G. fultoni* and *G. vernalis* sometimes had trials with tie outcomes.

agonistic trials, respectively, included grapple (Table 2). By contrast, no trials in *G. fultoni* and *G. vernalis* included grapple. In *G. fultoni* and *G. vernalis*, the aggressive behavior with the highest intensity score was typically aggressive song. In the post hoc analyses, *G. fultoni* and *G. vernalis* were not different in aggressiveness, and these two species had low levels of aggressiveness (Table 3). The two species with high levels of aggressiveness, *G. pennsylvanicus* and *G. rubens*, were not different in aggressiveness either. However, the two species groups differed significantly in aggressiveness in any combination (Table 3).

In bivariate ordinal regression analyses, contest duration was a significant factor affecting aggressiveness in cricket agonistic interactions, but age difference and size difference were not (Table 4). However, when trials with tie outcomes were excluded from the bivariate ordinal regression, contest duration did not significantly affect aggressiveness in cricket agonistic interactions (Table 4). Therefore, species seemed to be the only factor explaining the variation in aggressiveness in our study.

Table 3 Post hoc comparisons of aggressiveness between species

	<i>G. vernalis</i>	<i>G. pennsylvanicus</i>	<i>G. rubens</i>
<i>G. fultoni</i>	U=640, P=0.661	U=104, P<0.001	U=130, P<0.001
<i>G. vernalis</i>		U=140, P<0.001	U=175, P<0.001
<i>G. pennsylvanicus</i>			U=380, P=0.321

Mann–Whitney *U* tests were conducted between species pairs of *G. fultoni* (n=31), *G. vernalis* (n=43), *G. pennsylvanicus* (n=33), and *G. rubens* (n=26).

Table 4 Results of bivariate ordinal regressions for cricket aggressiveness

Variable	n	χ^2	df	P value	Nagelkerke R ²
Duration (all data)	133	27.902	1	<0.001	0.227
Duration (without tie outcomes)	117	166.797	1	0.354	0.009
Age difference	133	1.164	1	0.281	0.010
Size difference	133	178.444	1	0.481	0.004

The three least-aggressive categories were combined into one, which restricted the ordinal regression analyses to only one independent variable besides the intercept (see text).

Contest outcome and dynamics

To understand how the contest outcomes were determined for each species, we conducted logistic regression separately for each species. There were no significant factors affecting contest outcomes in *G. fultoni*, *G. vernalis*, and *G. rubens* (Table 5). In *G. pennsylvanicus*, however, size and age differences had significant effects on contest outcome (Table 5). Because insufficient sample size for each species might have prevented us from detecting relationships between contest outcomes and predictor variables, we pooled species based on levels of aggressiveness (see Table 3). When species with low levels of aggression, *G. fultoni* and *G. vernalis*, were pooled, the binary logistic regression analysis identified aggressiveness as the only significant factor affecting contest outcome (Table 6, *G. fultoni* and *G. vernalis*). However, the same analysis identified age and size differences as significant factors in species with high levels of aggressiveness, *G. pennsylvanicus* and *G. rubens* (Table 6, *G. fultoni* and *G. vernalis*).

In *G. fultoni* and *G. vernalis*, losers in 37 agonistic trials (out of 58 trials with clear contest outcomes) displayed withdrawal upon contact without displaying any aggressive behavior, but winners in 54 agonistic trials displayed aggressive behaviors with intensity score 3 (Table 2). However, the frequencies of aggressive behaviors with highest intensity scores were very similar between winners and losers in *G. pennsylvanicus* and *G. rubens* (Table 2). The size difference between winners and losers did not have significant effects on contest outcomes in agonistic trials for *G. fultoni* and *G. vernalis* (Table 6, *G. fultoni* and *G. vernalis*). In *G. pennsylvanicus* and *G. rubens*, winners had longer hind femurs and younger (-0.86 ± 3.97 days, mean \pm SD; Table 6, *G. pennsylvanicus* and *G. rubens*) than losers. Thus, RHP seems to affect contest outcomes in *G. pennsylvanicus* and *G. rubens*, whereas crickets that displayed aggressive behaviors with higher intensity scores won the agonistic contests in *G. fultoni* and *G. vernalis* regardless of RHP.

Table 5 Results of binary logistic regressions for each species

Variable	<i>B</i>	SE	Exp(<i>B</i>)	Wald	<i>df</i>	<i>P</i> value
<i>G. fultoni</i>						
Aggressiveness				5.683	3	0.128
Duration	0.002	0.004	1.002	0.279	1	0.597
Age difference	0.085	0.079	1.089	1.159	1	0.282
Size difference	0.869	0.606	1.002	2.057	1	0.152
<i>G. vernalis</i>						
Aggressiveness				2.465	2	0.292
Duration	-0.007	0.004	0.993	3.202	1	0.074
Age difference	0.234	0.219	1.263	1.141	1	0.285
Size difference	1.311	0.894	3.708	2.151	1	0.143
<i>G. pennsylvanicus</i>						
Aggressiveness				2.295	4	0.682
Duration	0.000	0.003	1.000	0.014	1	0.905
Age difference	-0.276	0.126	0.759	4.828	1	0.028
Size difference	0.782	0.348	2.186	5.045	1	0.025
<i>G. rubens</i>						
Aggressiveness				2.348	3	0.503
Duration	0.001	0.005	1.001	0.019	1	0.891
Age difference	-0.237	0.146	0.789	2.619	1	0.106
Size difference	0.881	0.470	2.414	3.511	1	0.061

G. fultoni (-2 log likelihood=33.704, $\chi^2=32.839$, $df=6$, $P<0.001$, Nagelkerke $R^2=0.661$, $n=48$); *G. vernalis* (-2 log likelihood=29.632, $\chi^2=64.636$, $df=5$, $P<0.001$, Nagelkerke $R^2=0.613$, $n=68$); *G. pennsylvanicus* (-2 log likelihood=68.628, $\chi^2=21.466$, $df=7$, $P<0.003$, Nagelkerke $R^2=0.375$, $n=66$); *G. rubens* (-2 log likelihood=49.740, $\chi^2=23.715$, $df=6$, $P=0.001$, Nagelkerke $R^2=0.481$, $n=52$). Trials with tie outcomes were excluded from the analysis. Aggressiveness was an ordinal variable with five categories (see Table 1). Species differed in the number of categories of aggressiveness exhibited, as did the degrees of freedom in these analyses.

The initiator effect in agonistic interactions occurs when the cricket that instigates the first aggressive behavior upon contact is more likely to emerge as the eventual winner of the contest (Jackson 1991; Chen et al. 2002). The initiator effect was evident in *G. vernalis* (one-sided binomial test, $P<0.001$), but not in *G. fultoni* ($P=0.304$), *G. pennsylvanicus* ($P=0.592$), and *G. rubens* ($P=0.340$).

We examined whether the four cricket species differed in the temporal structure of agonistic trials, which included the

beginning of a contest, the initiation of aggression, and the end of a contest. There were significant differences among species in the periods between the beginning and end of a contest ($F_{3,113}=3.260$, $P=0.024$; Table 7) and between the beginning of a contest and initiation of aggression ($F_{3,113}=3.909$, $P=0.011$). But there was no difference among species in the period between initiation of aggression and the end of a contest ($F_{3,113}=1.784$, $P\geq 0.154$). Contest durations were generally shorter in *G. pennsylvanicus* and

Table 6 Results of binary logistic regressions for the pooled data

Variable	<i>B</i>	SE	Exp(<i>B</i>)	Wald	<i>df</i>	<i>P</i> value
<i>G. fultoni</i> and <i>G. vernalis</i>						
Aggressiveness				10.614	3	0.014
Duration	-0.003	0.002	0.997	1.552	1	0.213
Age difference	0.089	0.074	1.093	1.450	1	0.229
Size difference	0.643	0.407	1.902	2.493	1	0.114
<i>G. pennsylvanicus</i> and <i>G. rubens</i>						
Aggressiveness				4.743	4	0.315
Duration	0.000	0.003	1.000	0.001	1	0.976
Age difference	-0.262	0.094	0.770	7.699	1	0.006
Size difference	0.806	0.276	2.239	8.517	1	0.004

G. fultoni and *G. vernalis* (-2 log likelihood=69.912, $\chi^2=90.898$, $df=6$, $P<0.001$, Nagelkerke $R^2=0.724$, $n=116$) and *G. pennsylvanicus* and *G. rubens* (-2 log likelihood=118.483, $\chi^2=45.100$, $df=7$, $P<0.001$, Nagelkerke $R^2=0.424$, $n=118$). Trials with tie outcomes were excluded from the analysis. Aggressiveness was an ordinal variable with five categories (see Table 1). Species differed in the number of categories of aggressiveness exhibited, as did the degrees of freedom in these analyses.

Table 7 The temporal structures of agonistic trials in four cricket species

Species	Tie	Contest duration	First contact to aggressive behavior (s)	First aggressive behavior to withdrawal (s)	Dominance display (%)
<i>G. fultoni</i> (24)	7	139±132.2	40±58.5	98.9±121.9	21
<i>G. vernalis</i> (34)	9	143±120.6	78±97.9	65±81.6	34
<i>G. pennsylvanicus</i> (33)	0	86±92.6	39±82.0	47±59.5	100
<i>G. rubens</i> (26)	0	72±76.4	13±27.6	59±74.6	81

Tie indicates that a trial yielded no winner or loser during the 10-min trial (see the text). The contest duration was the time between the first contact and withdrawal. The contest duration was divided into two periods: first contact to first aggressive behavior and first aggressive behavior to withdrawal. Aggressive song and rock body was a dominance display by a winner after the contest outcome was determined. The numbers in parentheses after species names indicate the sample sizes. Contest duration, time from first contact to first aggressive behavior and first aggressive behavior to withdrawal, and dominance display were based on trials excluding ties. The total sample size for each species was the number in parenthesis plus the number of ties.

G. rubens than in *G. fultoni* and *G. vernalis* because the former two species quickly initiated aggressive behaviors upon contact (Table 7).

Markov chain analyses

Analyses of the first-order Markov chain transitions revealed nonrandom temporal association between behavioral patterns in all four species (likelihood ratio test: $\Lambda=148$, $df=64$, $P<0.001$ for *G. fultoni* winners; $\Lambda=163$, $df=36$, $P<0.001$ for *G. fultoni* losers; $\Lambda=119$, $df=49$, $P<0.001$ for *G. vernalis* winners; $\Lambda=129$, $df=36$, $P<0.001$ for *G. vernalis* losers; $\Lambda=790$, $df=144$, $P<0.001$ for *G. pennsylvanicus* winners; $\Lambda=623$, $df=121$, $P<0.001$ for *G. pennsylvanicus* losers; $\Lambda=723$, $df=144$, $P<0.001$ for *G. rubens* winners; $\Lambda=780$, $df=121$, $P<0.001$ for *G. rubens* losers). The Markov chain analyses showed qualitatively different patterns of aggressive behaviors among cricket species (Fig. 1). *G. pennsylvanicus* and *G. rubens* showed antennal fencing, mandible flare, and grapple, but *G. fultoni* and *G. vernalis* never exhibited these behaviors. The highest probability of the first-order transition ($58.5\% \leq X \leq 78.2\%$) was from antennal fencing to mandible flare in both losers and winners of *G. pennsylvanicus* and *G. rubens*. The second highest probability of the first-order transition ($51.6\% \leq X \leq 71.7\%$) was from mandible flare to grapple for *G. pennsylvanicus* winners, *G. pennsylvanicus* losers, and *G. rubens* losers. In *G. rubens* winners, the frequency of the transition from mandible flare to grapple was the third highest. Thus, antennal fencing, mandible flare, and grapple seemed to be a tightly related sequence of behavior in these species. All four species displayed rock body, kick, and aggressive song, but *G. fultoni* and *G. vernalis* rarely exhibited other aggressive behaviors such as bite, lunge, and chase (which together comprised $\leq 0.79\%$ of the total behavioral transitions in these species).

When the outcomes of contests were determined, the losers withdrew upon antennal contact or body contact

while the winners typically displayed aggressive song and rock body in all trials of *G. pennsylvanicus*. This dominance display was also evident in 81% of the trials in *G. rubens*. However, the percentages of trials containing this dominance display were only 21% in *G. fultoni* and 34% in *G. vernalis* (Table 7). The dominance display in *G. fultoni* and *G. vernalis* typically consisted of aggressive song only. In agonistic interactions, the percentage of trials involving the performance of the dominance display comprised of aggressive song and rock body were significantly different among four species (likelihood ratio test= 62.644 , $P<0.001$, $df=3$, $n=117$).

There were almost no differences in the types of behavior exhibited by winners and losers in all four species (Fig. 1). However, the frequencies of behavioral transitions that were related to the determination of contests were markedly different in winners and losers. For example, behavioral transitions involving withdrawal were much more frequently displayed by losers than winners in *G. vernalis*, *G. pennsylvanicus*, and *G. rubens*. In *G. fultoni*, the behavioral transition from withdrawal to body contact was also more than twice as frequent in losers (7.30%) than winners (3.47%). Behavioral transitions involving chase were almost exclusively exhibited by winners in all four species.

Discussion

Our comparative study of cricket agonistic behaviors revealed that variation in aggressiveness was strongly related to what species a cricket belonged to. However, factors that might be indicative of RHP such as size difference or age difference did not explain the variation in aggressiveness. The most conspicuous difference in aggressiveness among species was in the presence or absence of grapple, the most aggressive behavior in cricket agonistic behaviors. Most agonistic interactions of *G. pennsylvanicus* and *G. rubens*

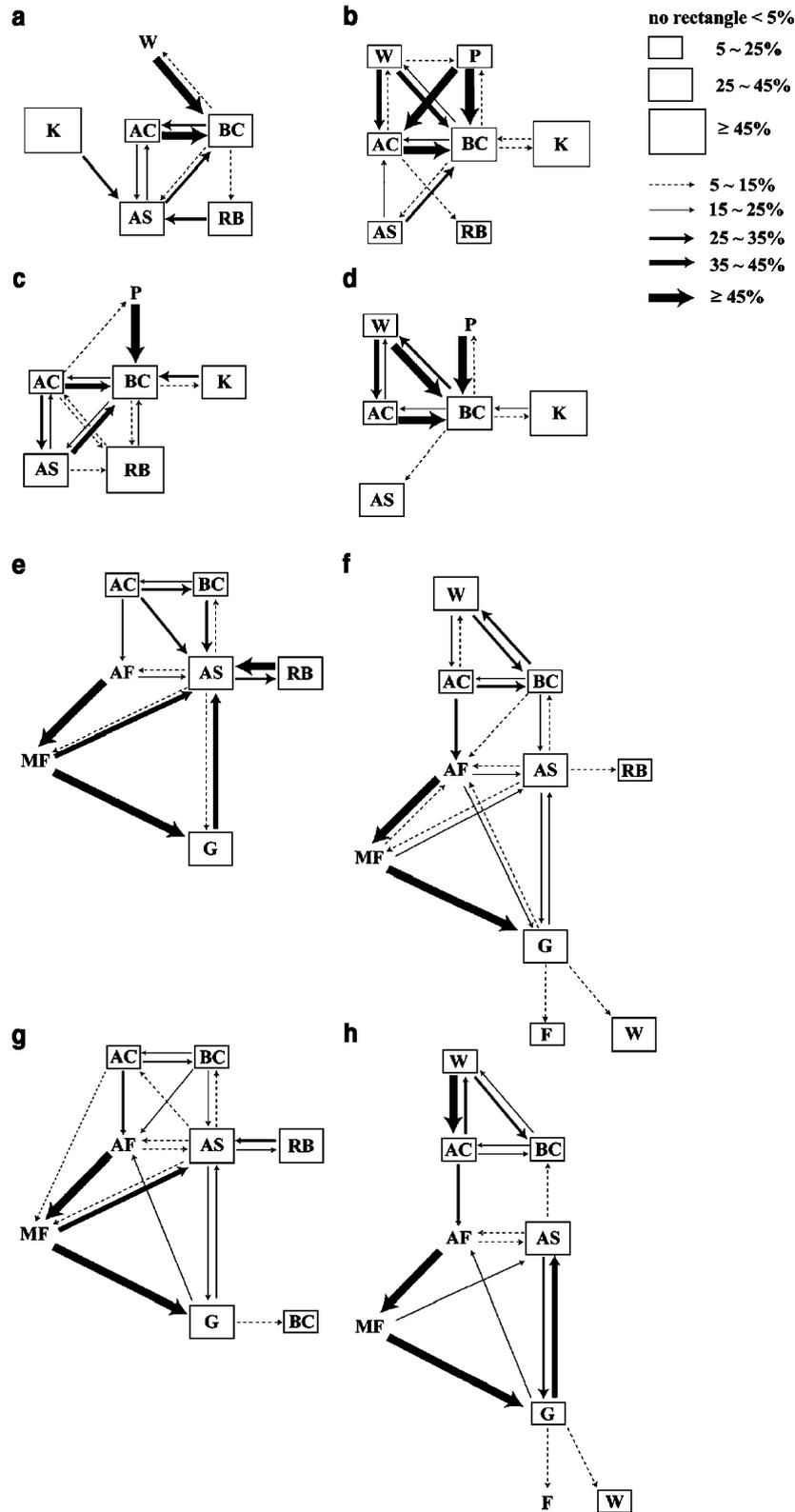


Fig. 1 Markov chain diagrams. **a** *G. fultoni* winners (the number of the total transitions, $n=317$), **b** *G. fultoni* losers ($n=411$), **c** *G. vernalis* winners ($n=346$), **d** *G. vernalis* losers ($n=331$), **e** *G. pennsylvanicus* winners ($n=946$), **f** *G. pennsylvanicus* losers ($n=642$), **g** *G. rubens* winners ($n=753$), and **h** *G. rubens* losers ($n=693$). A rectangle

denotes that the same behavior repeats itself in a row, and an *arrow* is a transition from one behavior (*the end of the arrow*) to the next (*the head*) with *thickness* being the probability. Behavioral transitions that were not significant with chi-square goodness-of-fit tests were not included in the Markov chain diagrams (see the “Materials and methods” section)

included grapple, but none of those of *G. fultoni* and *G. vernalis* did. The low levels of aggressiveness in *G. fultoni* and *G. vernalis* was unlikely to be influenced by the age structures of the four cricket species used in our study. Age ranges within which crickets show full repertoires of aggressiveness in the field were not known in the four species. However, the age ranges were largely overlapping among the four cricket species in our study. Thus, the variation in aggressiveness seemed to be explained by species differences, rather than differences in RHP. However, agonistic interactions of *G. fultoni* and *G. vernalis* should be further investigated in different contexts (e.g., conflict for food, see Adamo and Hoy 1995) to reveal their full repertoires of aggressive behaviors.

Why do species differ in aggressiveness? Male crickets use burrows for resting, attracting mates, and avoiding predation (Bateman 2000). In the field, males of *G. rubens* usually occupy burrows and defend them against conspecific males. They sometimes produce calling songs near the entrances of their burrows with their heads facing the entrances of the burrows or with parts of their anterior bodies still in the burrows (YJ, personal observation). When threatened, they escape into the burrows. Males of *G. pennsylvanicus* also use burrows but less frequently than males of *G. veletis* (Alexander and Bigelow 1960) or *G. rubens* (YJ, personal observation). They also use other natural cracks or crevices of various sorts. Alexander (1961) described a tendency of individuals to remain in restricted areas or to consistently return to specific spots as an indication of territoriality. By contrast, males of *G. fultoni* and *G. vernalis* seldom use burrows. They may use grasses or fallen leaves as a cover during calling or resting but do not establish long-term residence in burrows or cracks (Jang and Gerhardt 2006a). This difference in the tendency to use or defend burrows may explain variation in aggressiveness in the four field cricket species in this study. The aggressive behaviors leading to grapple are characteristic of defense of resources (e.g., burrows or mates) in various insects (see Choe 1994a, 1994b; Mason 1996; Greenfield and Minckley 1993). Other factors such as population density may also influence the variation in animal aggressiveness in field settings (Matthiopoulos et al. 2003).

In single-species analyses, size and age differences were critical factors influencing the outcome of contests for *G. pennsylvanicus*, but no significant factor was detected for the other cricket species. When data from agonistic trials were pooled, however, aggressiveness was the most important factor affecting contest outcome in the species, *G. fultoni* and *G. vernalis*, with low levels of aggressiveness. That is, crickets that displayed aggressive behaviors with higher intensity scores won the agonistic contests regardless of body size. In species, *G. pennsylvanicus* and *G. rubens*,

with high levels of aggressiveness, the factors that might be indicative of RHP such as size or age difference were significant for contest outcome. Accordingly, asymmetries in RHP estimated as age and size differences may not explain the results for *G. fultoni* and *G. vernalis* (see Parker 1974).

It is unclear what determines the variation in aggressiveness in agonistic interactions of *G. fultoni* and *G. vernalis*. Age difference, size difference, and contest duration were all unimportant (Table 1). We controlled for the effects of resource value such as food and shelter, but there may be unmeasured motivational differences between contests. For example, singing may affect physiological states in a way that tends to promote aggression in contests. That is, if a male produced calling songs before a contest, he may be

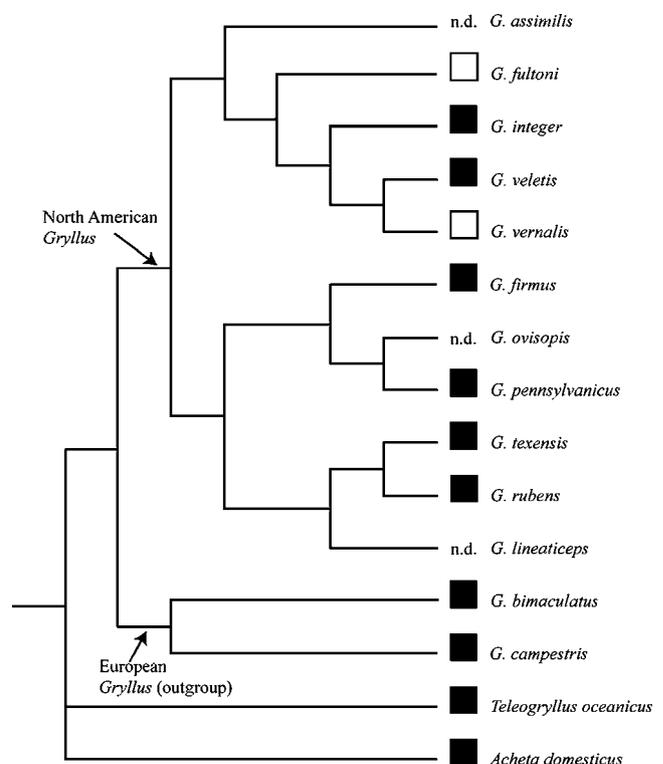


Fig. 2 Phylogeny of the North American *Gryllus* species with aggressiveness mapped onto it. The phylogeny was based on mitochondrial DNA sequences (Huang et al. 2000). *G. vernalis* was not included in the original phylogeny but was added later (Gray, personal communication). Cricket species with the escalated state of aggressiveness are represented by *solid squares*, and the ones with the deescalated state of aggressiveness are denoted by *open squares*. Cricket species for which aggressiveness was not examined are denoted by *n.d.* (no data). The presence of antennal fencing, mandible flare, and grapple indicates the escalated state of aggressiveness. References: *G. fultoni*, *G. vernalis*, *G. pennsylvanicus*, and *G. rubens* (this study); *G. firmus* (Alexander 1961); *G. integer* (Kortet and Hedrick 2007; Kortet, personal communication); *G. veletis* (Alexander 1961; personal observation); *G. texensis* (Adamo and Parsons 2006); *G. bimaculatus* (Adamo and Hoy 1995); *G. campestris* (Hissmann 1990); *Teleogryllus oceanicus* (Burk 1983; Burk, personal communication); *Acheta domesticus* (Hack 1997)

more aggressive than his opponent who did not sing before the contest. Male spotted antbirds (*Hylophylax n. naevioides*) that are more aggressive in male–male encounters also sing more (Hau et al. 2000) because testosterone regulates avian territorial behaviors such as song and aggression during the breeding season (Hunt et al. 1995). There is also the possibility of individual differences in aggressiveness. Most losers of *G. fultoni* and *G. vernalis* did not display highly aggressive behaviors during the trials. Rather, most losers withdrew from the agonistic interactions upon contact.

Phylogenetic analysis of cricket aggressiveness

When the aggression data are mapped onto the phylogenetic analysis based on mitochondrial DNA sequences (see Huang et al. 2000), it appears that the escalated state of aggressiveness may be the ancestral state in the North American *Gryllus* phylogeny (Fig. 2). Grapple is observed in at least three different clades of the North American *Gryllus* species. Furthermore, grapple is also observed in outgroup species such as *G. bimaculatus*, *Teleogryllus oceanicus*, and *Acheta domesticus* (see Huang et al. 2000). Among the species whose agonistic behaviors were studied, the deescalated state of aggressiveness occurred in only the one clade that contains *G. fultoni* and *G. vernalis*. The loss of the escalated state of aggression might have occurred independently in *G. fultoni* and *G. vernalis*. Alternatively, the loss of the escalated state of aggression may have evolved only once at the base of the *G. fultoni*–*G. integer*–*G. veletis*–*G. vernalis* complex and the escalated state of aggression may have then subsequently reappeared in *G. integer* and *G. veletis*.

The results of the phylogenetic analysis shift the question from why species differ in aggressiveness to why two species, *G. fultoni* and *G. vernalis*, have lost the tendency to perform grapple during agonistic encounters. We argue that the loss of a tendency to use or defend burrows in these two species may explain their deescalated state of aggressiveness. In the eastern United States, fallen leaves and grasses are readily available on the forest floor. During agonistic encounters, males of *G. fultoni* and *G. vernalis* may escape into nearby grasses or fallen leaves, instead of engaging in potentially costly grapples.

It is interesting to note that the agonistic interactions of *G. fultoni* and *G. vernalis* males were similar to those of *G. bimaculatus* females (Adamo and Hoy 1995). *G. bimaculatus* females, which do not exhibit territorial behavior, are capable of agonistically interacting with each other or with males, but their agonistic repertoire does not include grapple. Adamo and Hoy (1995) suggested that territoriality may explain this behavioral difference between *G. bimaculatus* males and females. Comparisons of agonistic behaviors be-

tween male and female *G. bimaculatus* (Adamo and Hoy 1995) and between species with high and low levels of aggressive behaviors in this study suggest the possibility that territoriality (i.e., burrow use) may accompany a series of behaviors which lead to grapple in the North American *Gryllus* crickets. Accordingly, we predict that any cricket species whose behavioral repertoire does not include territoriality may exhibit the deescalated state of aggressiveness.

Acknowledgments We are grateful to Yoonju Cho for the video analyses, to Mathew Zack for the statistical analyses, and to Susan Lappan and David Weissman for the critical comments. This paper was greatly improved by the critical reviews of anonymous referees. This work was supported financially by the University of Missouri Life Sciences Mission Enhancement Postdoctoral Fellowship and the Brain Korea 21 to YJ, by a U.S. National Science Foundation grant (IBN0091993) and a U.S. National Institute of Health grant (NIH R01 DC05760) to HCG, and by a Ewha Womans University research grant to JCC. The experiments in this study comply with the current law of the United States.

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