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A Quantitative Analysis of Agonistic Behavior in Juvenile American Lobsters (*Homarus americanus* L.)

Key Words
Crustacea
Agonistic behavior
Aggression
Ethogram
Escalated fighting
Assessment strategy
Jaccard's similarity measure
Matrix correlation
Principal components analysis

Abstract
In these studies a quantitative analysis of agonistic (fighting) behavior in lobsters in presented as a first step in our attempt to relate patterns of behavior to underlying neurobiological mechanisms. The agonistic behavior of juvenile American lobsters (*Homarus americanus L.*) was studied in laboratory tanks at the New England Aquarium. Using video analyses and statistical techniques: (1) an ethogram of agonistic behavior was constructed; and (2) the temporal structure of the behavior was identified. We demonstrated that fighting in juvenile lobsters proceeds according to strict rules of conduct. All animals exhibit six common behavioral patterns in a stereotypical manner. A temporal sequence of these patterns was evident, representing an increase in intensity during confrontations. The typical scenario of an encounter begins with extensive threat displays upon first contact, continues with periods of ritualized aggression and restrained use of the claws, and terminates in a brief session of unrestrained combat. Predictions of game theory (i.e. assessment strategies) provide a useful framework for the understanding of fighting in lobsters. The presence of a highly structured behavioral system may reduce the potential for damage in fights among conspecifics, and may prove useful in attempts to study the neurobiological causes of complex behavioral patterns such as aggression.

Introduction

The amines serotonin and octopamine may be intimately involved in agonistic behavior in lobsters [Kravitz, 1988]. The present investigation stems from observations that postural components of dominance, closely resembling those seen in lobsters during agonistic encounters, are triggered by injection of two amines; serotonin injection generates stances similar to that of dominant animals, octopamine injection results in a posture commonly seen in subdominant animals [Livingston et al., 1980]. The individual serotonin- and octopamine-containing neurons likely to be important in postural regulation have been identified in the ventral nerve cord of lobsters [Beltz and Kravitz, 1987; Ma et al., 1992; Schneider et al., 1993], and the physiological properties of the serotonin-containing cells have been studied extensively. These neurons serve as 'gain-setters', amplifying the output of circuitries involved in generating the elevated stance characteristic of dominant animals [Ma et al., 1992].

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The next challenging phase of these studies is to attempt to relate the neurobiological studies to behavior. A first-order question is whether linkages exist between the functioning of amine neurons and the appearance or maintenance of agonistic behavioral patterns. To begin studies exploring such linkages, it is necessary to quantify the behavior. Quantification would allow us (1) to utilize animals of precisely defined behavioral status in neurophysiological, biochemical and molecular genetic analyses of neuronal function; and (2) to utilize pharmacological reagents to manipulate the concentration, metabolism, or actions of amines, in order to search for associated changes in behavior.

In lobsters, as in other decapod crustaceans, the ability to dominate conspecifics plays a vital role in their biology. Lobsters, therefore, are good animals for studies of agonistic behavior. Dominance behavior has been studied in crayfish [Bruski and Dunham, 1987], stomatopods [Caldwell, 1979], prawns [Evans and Shehadi-Moacdieh, 1988], spiny lobsters [Cobb, 1980], and the American lobster used in our studies [Scrivener, 1971]. In American lobsters, agonistic encounters are used for the acquisition and defense of shelters, which are necessary to avoid predation, and to gain access to food and mates [Hyatt, 1983; Atema, 1986]. The acquisition of protective shelter is particularly important for early juveniles, because attacks by benthic predators are common during the first year of life [Lavalli and Barshaw, 1986; Barshaw and Lavalli, 1988]. Many aspects of the biology of juvenile lobsters may be interpreted as adaptations to this quest for shelter. Young animals usually occupy solitary burrows [Barshaw and Bryant-Rich, 1988] where plankton, drawn into the burrows via self-generated currents, is an important source of food [Lavalli and Barshaw, 1989; Lavalli and Factor, 1992]. Fighting behavior inevitably results when two juveniles are introduced into a common space. Staged encounters between lobsters lead to fights of varying length and intensity. Asymmetries in size, sex, molt state, physical condition, and prior experience serve as predictors of success in these contests [review in Atema and Cobb, 1980]. In general, with decapods, confrontations are more likely to escalate if combatants are closely matched in these factors [c.f. Hazlett, 1968; Scrivener, 1971; Caldwell and Dingle, 1979; Evans and Shehadi-Moacdieh, 1988]. Physical combat often is preceeded by extensive and conspicuous threat displays or ritualized combat, i.e. routines during which the use of damaging weapons is avoided or restricted to harmless maneuvers [Immelmann and Beer, 1989]. Game Theory predicts that agonistic behavior may provide an assessment of an opponents fighting ability [Parker, 1974] while effectively reducing the risk of injury to both combatants. A close examination of aggressive encounters in lobsters, where opponents easily can inflict injury, may provide insights into the behavioral mechanisms that effectively reduce the dangers of weapons such as claws.

The goal of this study is to provide a detailed account of the fighting behavior of juvenile lobsters. Such an account may serve as a general framework for further neurobiological investigations into the proximate causation of this behavior. With the use of quantitative methodology we derive an ethogram of the agonistic behavior of juvenile lobsters, identifying different aspects of fighting behavior in this species. First we dissociate agonistic behavior into a series of distinct, fundamental elements (i.e. basic motor actions), the occurrence of which can be identified reliably by different observers. Next we identify the behavioral patterns (combinations of the fundamental elements) characteristic of aggressive interactions and summarize these results in an ethogram. Then we consider the temporal structure of the agonistic patterns during such encounters. Finally, we evaluate the stability and similarity of the behavior among different animals, or in the same animal on different days.

Materials and Methods

American lobsters of known age, sex, and molt stage are raised under controlled environmental conditions at the Edgerton Research Laboratory, New England Aquarium (Boston, Mass., USA). They are maintained at steady temperatures (15–20 °C) with a light:dark cycle of 14 h light: 10 h dark in a large sea-water system. Egg-bearing lobsters are collected by local fishermen in the summer and fall of the year and kept in communal tanks at the aquarium at low temperature (10 °C) to slow the rate of embryonic development. On a six week cycle, single egg-bearing females are transferred to higher temperature tanks (20–25 °C) for hatching. Larvae are hatched in the laboratory and raised communally until Stage IV when they begin to settle to the substrate. At that time, animals are transferred to individual plastic cups supplied with their own water inflow and containing a small shelter. They are fed a mixed diet of mussels, clams, squid, shrimp, brine shrimp, and fish. All animals used in this study were juveniles without previous agonistic experience (for descriptive statistics see table 1). Carapace length ranged from 2–5 cm and animals were between 6 months to 1.5 years old.

Experimental Conditions

Observations were conducted in tanks (45 × 20 cm) partitioned by a removable divider into two adjacent compartments, each of which contained a small shelter. Ten pairs of animals which had not previously fought with a conspecific were closely matched in terms of size and molt state, to reduce overt asymmetries and to foster conditions for escalated fights. Only animals in molt states C, D0 and D1 [Aiken, 1973] were used. Matched pairs of animals were placed into the observation tank, one lobster per compartment. Following an acclimation

Table 1. Weight (in grams) and sex of juvenile animals used in this study and summary information on the interaction of each pair on days 1 and 2

Pair	Animal A		Animal B		Day 1				Day 2			
	weight	sex	weight	sex	N	N_F	bouts	win	N	N_F	bouts	win
1	2.63	M	2.63	F	473	99	1	B	180	3	1	B
2	2.03	F	2.12	F	269	7	1	B	509	183	2	B
3	9.78	M	9.91	M	395	140	1	B	252	0	1	B
4	8.08	F	7.66	F	306	62	1	A	249	1	1	A
5	3.62	F	3.12	F	377	61	2	A	252	0	1	A
6	13.62	F	13.41	M	720	164	5	B	720	108	4	B
7	7.67	F	6.90	M	479	6	2	A	263	2	1	A
8	14.52	F	16.11	M	720	161	7	B	521	76	3	B
9	13.35	F	12.82	M	720	345	3	A	325	78	1	A
10	22.50	M	22.00	M	468	90	3	A	720	279	7	A

N indicates the total number of observation intervals quantified, and N_F the number of observation intervals in which both animals exhibited approach-oriented agonistic behavior.

period of at least 24 h, the divider and the shelters were removed. All subsequent interactions were recorded on videotape, using a video camera (Panasonic WV-3260) that placed a time display on the videotape. Encounters began between 09:00 and 12:00 h, and continued for 1 h or until one animal retreated consistently for 20 min. Preliminary observations suggested that a 20 min cut-off time reduced the chances of physical damage to losers who failed to withdraw from winners after repeated tailflips to escape. Test studies showed also that once established, a dominance relationship was rarely reversed or challenged for the next hour if it had been established for 20 min. At the end of an encounter, the recording was stopped and the opponents were separated into their original compartments with the divider. The following day the same two animals were subjected to an identical experimental protocol.

Collection of Data

'Aggression' has been defined in various ways in both the popular and scientific literature [Johnson, 1972]. In this study, we use the more precise term 'agonistic behavior'. Agonistic behavior is the set of patterns that share a common function: adjustment to situations of conflict among conspecifics. It includes threat, submission, chases, and physical combat [Drickamer and Vessey, 1982]. Two categories of agonistic behavior are distinguished: (1) approach-oriented agonistic behavior (approach behavior) – all patterns of agonistic behavior that direct an animal towards an opponent; and (2) avoidance-oriented agonistic behavior (avoidance behavior) – all patterns that steer an animal away from an opponent.

The behavior was quantified from the videotape recordings in the following way. The total observation period, monitored separately for each animal, was divided into five-second segments; during each of these segments the occurrence of 17 behavioral variables was recorded. For every interval we noted whether an animal was within one body length of the other and whether 16 fundamental motor actions were present or absent (table 2). The success of such an analysis depends on the selection of an appropriate set of variables. In these experiments the selection was based on previous descriptions of agonistic behavior in American lobsters [Scrivener, 1971] and crayfish [Dingle, 1969; Bruski and Dunham, 1987] and on observations of a preliminary series of 50 interactions conducted in our laboratory. In choosing the variables, we attempted to dissociate behavioral actions into discrete elements that could be reliably and consistently identified by different observers. Staged encounters in lobsters usually feature one or several periods in which both animals exhibit approach-oriented agonistic behavior. Such periods are called bouts and are separated by periods of no contact or of avoidance behavior by one or the other of the combatants [Scrivener, 1971; Atema and Cobb, 1980]. A bout begins when both lobsters initiate approach behavior. The end of a bout is defined operationally as the time following which neither animal shows approach behavior for at least 5 min. The analysis of fighting behavior is restricted to contested periods (the bouts). In several experiments, a dominance relationship was established in less than 4 min (table 1). Such short encounters were excluded from the analysis. Hence, analyses were conducted on a restricted data set of 3,711 observation intervals containing data for 18 animals.

Identification of Agonistic Behavioral Patterns

Certain of the 17 variables we measure may occur together in distinct groupings that comprise more complex behavioral patterns. Those actions that repeatedly occur together in single 5 s intervals may be associated to form such patterns. To evaluate the co-occurrence of several variables statistically, two independent quantitative approaches were used: (i) Jaccard's similarity measure [Procedure PROXIMITIES: SPSS Inc., 1988] and (ii) principal components analysis [Procedure FACTOR: SPSS Inc., 1988].

(i) Jaccard's similarity measure (i.e., the similarity ratio) is designed to detect relationships among 'items' based on the presence or absence of a set of variables (a, b).

$$\text{Jaccard similarity measure }(x, y) = \frac{\sum \text{(a and b)}}{\sum \text{(only a)} + \sum \text{(only b)} + \sum \text{(a and b)}}$$

Table 2. The presence or absence of the following behavioral elements was quantified for each five second interval

Approach	A lobster advances towards an opponent slowly (< 1 body length/sec) reducing the distance between the animals to less than 1 body length
Lunge	Similar to approach, but advance towards the opponent is rapid (> 1 body length/sec)
Retreat	An animal moves or turns away from an opponent
Tailflip	An escape response during which a rapid contraction of the abdomen propels a lobster backwards
Body up	The body is raised high above the substrate on fully extended walking legs
Claw up	One or both claws are lifted high above the horizontal and extended laterally
Claw down	One or both claws are pointed straight down towards the substrate
Claw touch closed	An animal touches the opponent with closed claws
Claw touch open	A lobster touches an opponent with open claws
Claw grasp	A lobster uses its claw to grab an appendage of the opponent
Claw rip	A rapid motion in which an animal grasps the opponent and pulls at it quickly
Claw strike	A lobster strikes towards the opponent with one or both of its claws
Pushing, pulling	An animal attempts to displace the other through pushing or pulling using walking legs and pleopods
Antennae up	Both antennae are pointed straight up and away from the opponent
Antennae tap	In a single motion, an antenna is rapidly swept downwards over the anterior portion of the thorax of the opponent
Antennae whipping	One or both antennae vigorously and repeatedly lash the opponent in rapid sequence

Here it is used to identify frequent co-occurrences of two motor actions, by comparing the likelihood that the actions occur together to the likelihood that each action occurs without the other in a given observation interval. The similarity measure ignores instances in which both actions are absent from the data set. A similarity measure is calculated for every possible pair of variables, and the measures are summarized in the form of a similarity matrix.

(ii) Principal components analysis (PCA), is a method of factor analysis that has been shown to be an appropriate tool for behavioral investigations [Lisak and Roth, 1988; Temoshok et al., 1988; Bouchard and Lynch, 1989]. We used this form of factor analysis as an alternative to Jaccard's similarity measure: (1) to identify the underlying components or 'factors' that explain the correlation between sets of variables; (2) to summarize a large number of variables with a smaller number of 'derived' variables; and (3) to determine the number of dimensions required to economically represent a complex set of variables [Sokal and Rohlf, 1981; SPSS Inc., 1988]. The binary representation of behavioral variables we use in these studies cannot show a normal distribution. Despite this limitation, principal components analysis is used since it is a robust statistical procedure that is insensitive to violation of this requirement. The Kaiser-Meyer-Olkin measure of sampling adequacy and Bartlett's test of sphericity also were utilized to evaluate the applicability of principal components analysis. A low value (< 0.5) for the Kaiser-Meyer-Olkin measure of sampling adequacy indicates that a factor analysis may not be appropriate, since correlations between pairs of variables cannot be explained by the other variables [Kaiser, 1974]. Furthermore, a factor analysis may not be valid unless Bartlett's test of sphericity proves significant, thereby indicating that the variables correlate sufficiently well with each other [SPSS Inc., 1988]. Our data justify the use of this type of analysis, since Bartlett's Test of Sphericity ($X^2 = 6383.2$; $p << 0.001$) was significant and the Kaiser-Meyer-Olkin measure of sampling adequacy, although low, was in an acceptable range (total matrix sampling adequacy = 0.62).

The identification of non-random temporal associations between the behavioral patterns is of interest in order to detect whether there is a sequence to the behavior and to determine whether the levels of intensity escalate during a fight. The analysis of patterns of change over time has been summarized in a recent review [Gottman and Roy, 1990]. In these studies we construct transition matrices by tabulating all instances in which one behavioral pattern (i.e., one state) leads to another. All of the observation intervals also are assigned to 1 of 5 categories representing a sequence of increasing intensity of fighting: (1) no agonistic behavior; (2) no physical contact; (3) physical contact but claws not used to grasp the opponent; (4) claws grasp the opponent; and (5) unrestrained use of claws with striking and ripping. To evaluate whether the transitions between the states or the fighting intensities are random, or whether certain transitions are more or less likely to occur than others, likelihood-ratio tests (G-statistics) are applied. In the cases in which the overall matrix shows significance, cell-wise examinations (Freeman-Tukey deviates) are performed to identify the cells that brought about the significance.

The different matrices (i.e. Jaccard's similarity matrix, the matrix of factor loadings from the principal components analysis, the transition matrix) represent 'snapshots' of the patterns present in agonistic behavior, and a separate snapshot is derived for each animal on each day. The similarity in fighting behavior between animals (or in the same animal on different days) can be evaluated statistically using Mantel Matrix procedures to test for homogeneity of the matrices. This nonparametric technique compares two or more matrices [Mantel, 1967; Schnell et al., 1985] and evaluates the statistical association between the calculations of interanimal distances based on one behavioral characteristic with that calculated from a second behavioral characteristic.

Results

Behaviorally naive juvenile lobsters readily engage in agonistic encounters. Although no two encounters are identical, a general description is possible (fig. 1). When animals approach each other, both engage in a variety of threat

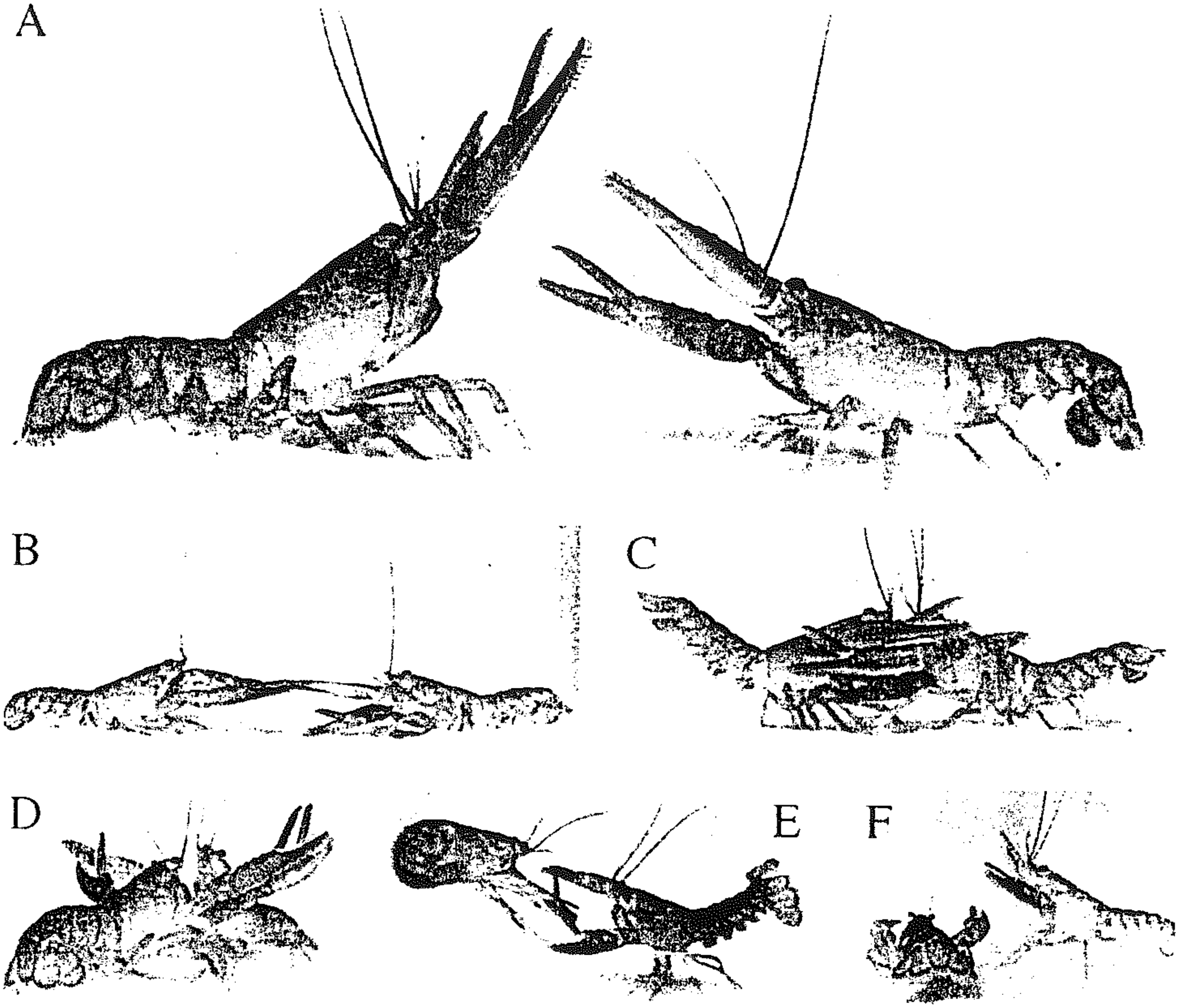
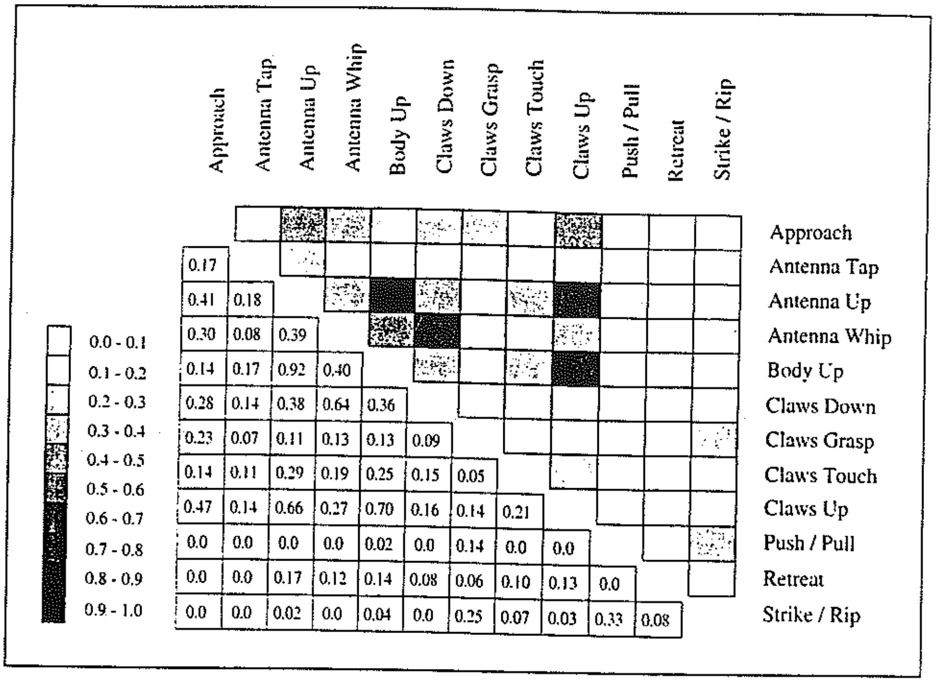


Fig. 1. Digitized images of behavior patterns observed during agonistic interactions of American lobsters: **A** Meral spread: **B** and **C** wrestling: **D** reaching: **E** retreat: **F** dominant and sub-dominant stances.

displays with little physical contact: the most common display is 'meral spread' [Dingle. 1969]. In a highly ritualized aspect of lobster fighting. animals lash each other with their antennae ('do-si-do'). Next comes a stage featuring restrained physical contact in which opponents touch each other with claws that are mostly kept in a closed position. They repeatedly stretch their claws far apart as if measuring each others span ('reaching'). If the fight continues. the opponents lock claws and attempt to displace each other by pulling or pushing. They also attempt to lift their opponent off the substrate and turn it onto its back. In many cases. the outcome of the encounter is decided during these initial stages. thereby avoiding the physical injury that can result from the later. more violent stages of conflict. If no decision is reached. however. the intensity of the fight increases dramatically. The last phase is usually short in duration. with both animals making extensive and unrestrained use of their claws. They grasp the antennae. chelipeds. or legs and attempt to tear those appendages from the opponent. In juvenile animals this ripping motion is amplified by the use



	Approach	Antenna Tap	Antenna Up	Antenna Whip	Body Up	Claws Down	Claws Grasp	Claws Touch	Claws Up	Push / Pull	Retreat	Strike / Rip
Approach												
Antenna Tap	0.17											
Antenna Up	0.41	0.18										
Antenna Whip	0.30	0.08	0.39									
Body Up	0.14	0.17	0.92	0.40								
Claws Down	0.28	0.14	0.38	0.64	0.36							
Claws Grasp	0.23	0.07	0.11	0.13	0.13	0.09						
Claws Touch	0.14	0.11	0.29	0.19	0.25	0.15	0.05					
Claws Up	0.47	0.14	0.66	0.27	0.70	0.16	0.14	0.21				
Push / Pull	0.0	0.0	0.0	0.0	0.02	0.0	0.14	0.0	0.0			
Retreat	0.0	0.0	0.17	0.12	0.14	0.08	0.06	0.10	0.13	0.0		
Strike / Rip	0.0	0.0	0.02	0.0	0.04	0.0	0.25	0.07	0.03	0.33	0.08	

Fig. 2. Jaccard's similarity matrix for lobster #10 on day 1. The lower left triangle contains similarity measures for all possible pair-wise comparisons. The probability that any two actions occur together within an observation interval (five seconds) is also indicated graphically by the darkness of the corresponding box in the upper right triangle.

of short, upward directed tail flips, which keep the animals in close proximity. After a time, one animal retreats, and sometimes remains in place in a corner of the tank. The other animal initiates further bouts until the 'loser' consistently retreats from the advances of the 'winner'. On average the bouts last approximately 10 min on the first day (114 five-second observation intervals; mean ± SEM = 568 ± 157 s), and 6 min on the second (73 intervals; mean ± SEM = 365 ± 151 s) (table 1). This difference was not statistically significant (Wilcoxon $\chi^2 = 1.85$ or Normal Quantiles $\chi^2 = 2.02$; df = 1; $p > 0.05$).

Classification of the Agonistic Behavioral Patterns

A matrix of Jaccard's similarity measures is calculated for each animal on each day summarizing the probability of finding any two actions co-occurring within a five-second observation interval (fig. 2). As can be seen in the matrix, the actions of lifting the claws, standing in an elevated posture and pointing the antennae up often appear together and may comprise a group, while pointing the antennae up and lowering the claws rarely co-occur. Principal components analysis (PCA) of the same data set indicates that certain motor actions appear to be grouped (fig. 3). Six commonly observed behavioral patterns can be derived from the PCA, cumulatively accounting for 71% of the data. A detailed description of these 6 behavioral patterns is produced by the matrix of factor loadings after Varimax rotation (fig. 3). Each factor loading represents a measure of correlation between an original variable (e.g. body up) and a derived factor (e.g. behavioral pattern or factor I). A high positive value[1] indicates that a motor action is part of the composite pattern, whereas a high negative value suggests that the motor action rarely occurs in the derived behavioral pattern. For example, in the first behavioral pattern (factor I) an animal stands high on its walking legs, the antennae are pointing straight up and back, and the claws are held elevated and may touch the opponent. This pattern of behavior, called meral spread, has been observed previously in lobsters and in other decapod crustaceans [Dingle, 1969; Scrivener, 1971]. In the behavioral pattern described by factor II (wrestling), animals grasp the claws of the opponent and attempt to displace each other by pushing or pulling, while maintaining the antennae pointing straight up and back. The third pattern, do-si-do, is characterized by one animal standing high on its walking legs, pointing its claws straight downward, approaching the opponent, and whipping the opponent with its antennae, while the other animal retreats in a lowered posture. The animals may reverse roles in this interesting 'dance step'. The other behavioral patterns are represented by single quantified variables: (IV) retreat; (V) antenna tap; and (VI) strike and rip.

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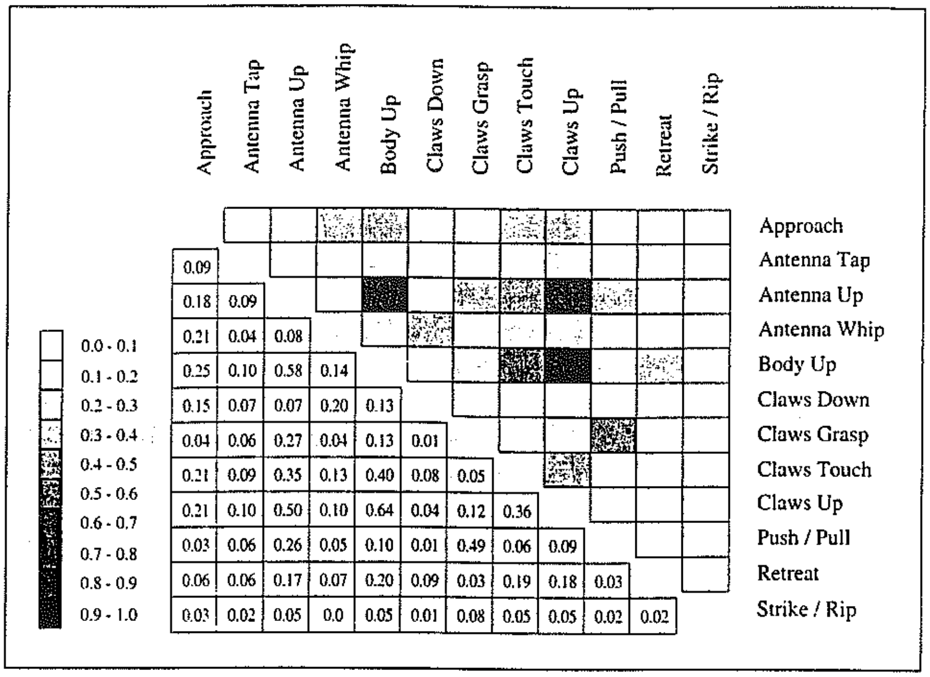
[1] Ratings for the magnitude of the factor loadings follow Comrey (1973): A factor loading of 0.71 or above can be considered excellent, 0.63 is very good, 0.55 is good, 0.45 is fair, and 0.32 is poor. The square of the factor loading represents a measure of the amount of variance in common between the variable and the factor.

Fig. 3. Factor loadings (Varimax rotated) of each variable on principal component axes and the cumulative variance explained by each axis. Large loadings (highlighted) indicate variables that contribute highly to a specific behavior pattern. For this analysis several variables were grouped: approach (approach, lunge), retreat (retreat, tailflip), claw touch (claw touch open, claw touch closed), strike/rip (claw strike, claw rip).

	Factors					
VARIABLE	I	II	III	IV	V	VI
Body Up	0.821	0.12	0.13	0.02	0.05	0.03
Antenna Tap	0.00	0.02	-0.06	-0.03	0.978	-0.01
Antenna Up	0.779	0.795	-0.09	0.13	0.06	0.01
Antenna Whip	0.01	0.02	0.737	-0.13	-0.22	-0.10
Claws Up	0.958	0.14	-0.31	-0.12	-0.03	-0.04
Claws Down	-0.24	-0.02	0.877	0.25	0.15	0.10
Claw Grasp	0.08	0.872	0.01	-0.03	0.01	0.16
Push/Pull	0.00	0.857	0.01	0.00	-0.01	-0.12
Approach	0.15	-0.20	0.332	-0.546	0.04	-0.05
Retreat	0.10	-0.08	0.16	0.842	-0.01	-0.06
Claw Touch	0.577	-0.05	0.01	0.07	-0.05	0.06
Strike/Rip	0.09	0.05	-0.02	-0.03	-0.01	0.975
Variance (%)	20.1	14.7	11.2	8.7	8.3	8.0
Cum. Variance (%)	20.1	34.8	46.0	54.7	63.0	71.0
Behavior Pattern	'Meral Spread'	'Wrestling'	'Do-si-do'	'Retreat'	'Antenna Tap'	'Strike/ Rip'

Indiv./Day	2/1	3/2	4/2	5/1	6/1	7/1	8/1	9/1	10/1	11/1	11/2	12/1	12/2	15/1	16/1	16/2	17/1	17/2	18/1	19/1	19/2	20/1
1/1	***	*	*	***	**	***	ns	ns	ns	ns	*	ns	*	***	ns	*	***	**	***	**	**	**
2/1		**	**	***	***	***	***	***	***	***	***	**	***	***	***	**	***	***	***	***	***	***
3/2			***	**	*	*	ns	ns	ns	ns	***	ns	***	*	ns	***	***	ns	***	*	***	ns
4/2				**	*	*	*	*	*	ns	***	ns	***	*	ns	***	***	*	***	*	***	ns
5/1					***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
6/1						***	***	***	***	***	***	***	**	***	***	**	***	***	***	***	**	***
7/1							***	***	***	***	***	**	**	***	***	**	***	***	***	***	***	***
8/1								***	***	***	***	**	**	***	***	**	***	***	***	***	**	***
9/1									***	***	***	ns	***	***	***	***	***	**	***	***	***	***
10/1										**	***	**	**	***	***	***	***	**	**	***	**	***
11/1											***	***	**	**	***	**	***	**	***	***	**	***
11/2												ns	***	***	***	***	***	**	***	***	***	***
12/1													ns	***	**	ns	***	**	**	***	ns	***
12/2														***	***	***	***	***	***	***	***	***
15/1															***	**	***	***	***	***	**	***
16/1																ns	***	***	***	***	*	***
16/2																	***	*	***	**	***	**
17/1																		***	***	***	***	***
17/2																			***	***	**	***
18/1																				***	***	***
19/1																					***	***
19/2																						***

Fig. 4. Levels of significance for all possible comparisons of the fighting behavior observed during different experiments. Mantel's matrix procedures were performed to relate the behavior pattern matrix (BPM) of an individual on a particular day, with its BPM on other days (e.g., the 11/1 to 11/2 comparison) as well as to the BPM of all other individuals on any days (e.g., the 1/1 to 2/1 comparison). Data for 1/2 (i.e. individual 1 – animal A of pair 1 – on day 2), 2/2 (i.e. individual 2 – animal B of pair 1 – on day 2), 3/1, 4/1, 5/2, 6/2, 7/2, 8/2, 9/2, 10/2, 13/1, 13/2, 14/1, 14/2, 15/2, 18/2, 20/1 and 20/2 were excluded due to small sample size. Twenty-four comparisons were non-significant (ns). 19 comparisons were significant at $0.05 \geq p > 0.01$ (*), 42 were significant at $0.01 \geq p > 0.001$ (**), and 168 at $0.001 \geq p$ (***). The expected outcome for 253 comparisons at $P \geq 0.05$ is 13 tests significant by chance alone. The actual total of 229 significant comparisons is considerably higher than that, indicating strong homogeneity among all behavior matrices.



	Approach	Antenna Tap	Antenna Up	Antenna Whip	Body Up	Claws Down	Claws Grasp	Claws Touch	Claws Up	Push / Pull	Retreat	Strike / Rip
Approach												
Antenna Tap	0.09											
Antenna Up	0.18	0.09										
Antenna Whip	0.21	0.04	0.08									
Body Up	0.25	0.10	0.58	0.14								
Claws Down	0.15	0.07	0.07	0.20	0.13							
Claws Grasp	0.04	0.06	0.27	0.04	0.13	0.01						
Claws Touch	0.21	0.09	0.35	0.13	0.40	0.08	0.05					
Claws Up	0.21	0.10	0.50	0.10	0.64	0.04	0.12	0.36				
Push / Pull	0.03	0.06	0.26	0.05	0.10	0.01	0.49	0.06	0.09			
Retreat	0.06	0.06	0.17	0.07	0.20	0.09	0.03	0.19	0.18	0.03		
Strike / Rip	0.03	0.02	0.05	0.0	0.05	0.01	0.08	0.05	0.05	0.02	0.02	

Fig. 5. A composite matrix of Jaccard's similarity measures for all individuals and days combined. The lower left triangle contains similarity measures for all possible pairwise comparisons. The probability that any two actions are found together within an observation interval (5 s) is indicated graphically by the darkness of the corresponding box in the upper right triangle.

Similarities in Agonistic Behavior between Different Animals and within Animals on Different Days

If agonistic behavior in lobsters is composed of stereotypical components shown by all animals in all encounters, then the patterns seen on similarity matrices should bear a statistically significant relationship to each other. Mantel's Matrix Procedure identified highly significant levels of homogeneity among the matrices (fig. 4). Of 253 comparisons made, 229 were significant at $p \leq 0.05$ (chance alone would predict that 13 of the 253 comparisons would be significant at that level). Since the matrices were homogeneous, the data of all the animals were grouped together to construct an overall similarity matrix (fig. 5). The grouped data bear a striking similarity to the single animal data, providing a further demonstration of the stereotypy in these patterns of behavior.

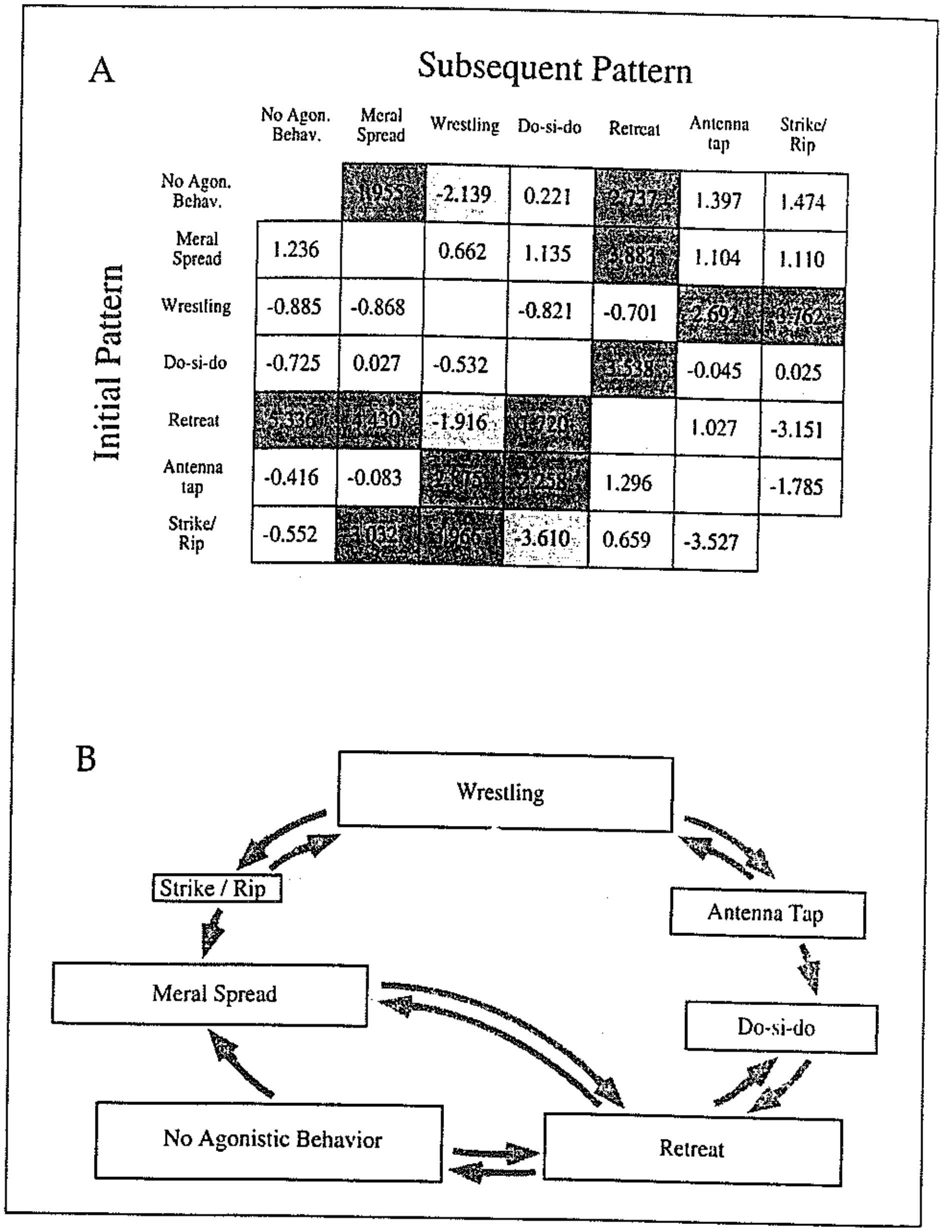
The Sequential Analysis of Agonistic Behavior

A likelihood-ratio test (G-statistic) on the transitions between the 6 behavioral patterns proved highly significant (likelihood ratio $\chi^2 = 255.5$; df = 25; $p < 0.001$), suggesting the existence of a clear temporal sequence to the fights. Subsequent cell-by-cell analysis identified the transitions primarily responsible for the overall significance (fig. 6b). For example, animals often go from no agonistic behavior to displays like meral spread, but they rarely switch from no agonistic behavior to wrestling.

Most behavioral patterns do not result in damage to an opponent. An analysis of behavioral intensities showed that agonistic encounters predominantly were made up of elaborate threat displays, accounting for 84% of the fighting time. This included observation intervals without physical contact (1,139 intervals, 31%), ritualized fighting without grasping the opponent (1,029 intervals, 28%), and grasping the opponent without striking and ripping (918 intervals, 25%). Animals made unrestrained use of their claws for striking and ripping during only 4% of the fighting time (147 intervals). A sequential analysis of intensity categories also proved significant and demonstrated an increase in intensity during an encounter. A subsequent cell-wise examination demonstrated that transitions are more likely to occur between categories of similar intensity and less likely to occur between categories that are dissimilar (fig. 7).

Discussion

The application of multivariate statistical techniques has allowed us to generate a quantitative description and characterization of agonistic behavior in lobsters. In a probabilistic sense, several features of aggressive encounters are shown to be similar for all animals on all days. This allows us to make claims concerning their 'typical' or 'usual' appearance and enhances the utility of the behavioral assay



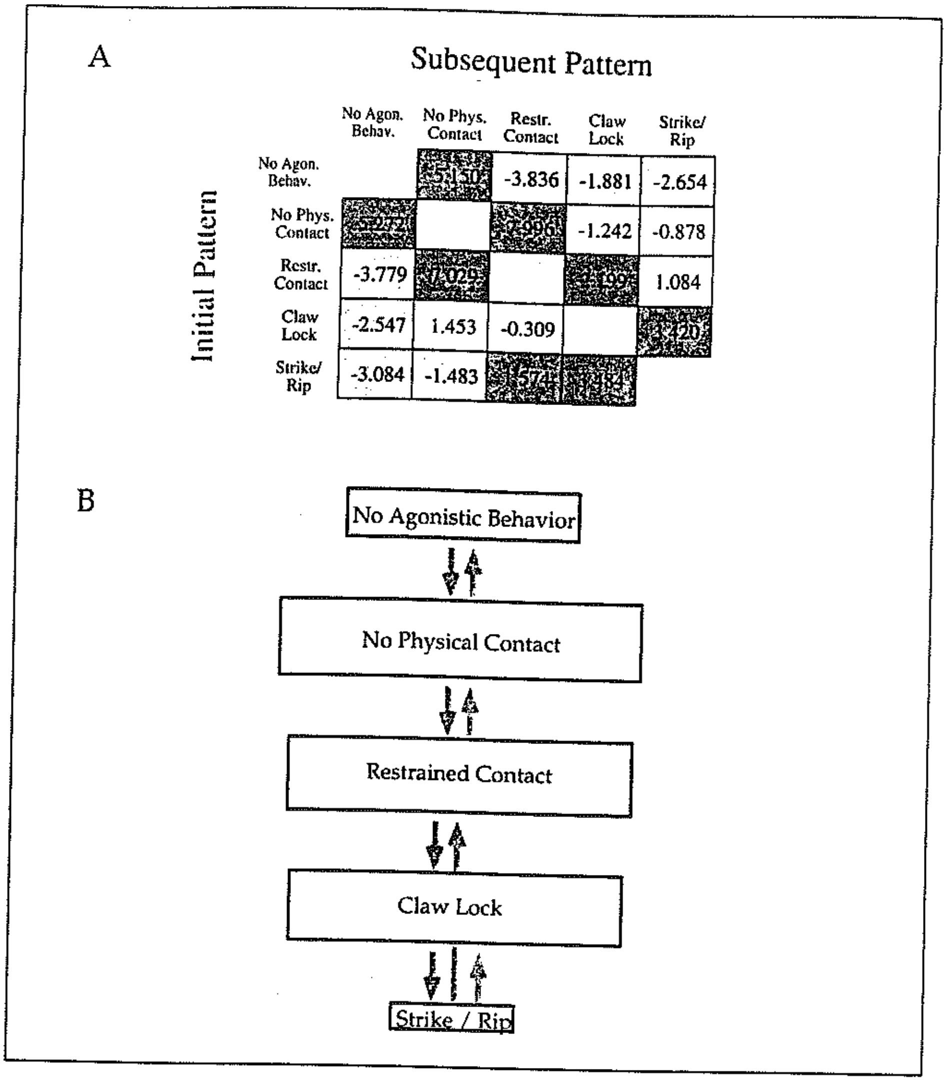
	No Agon. Behav.	Meral Spread	Wrestling	Do-si-do	Retreat	Antenna tap	Strike/ Rip
No Agon. Behav.		1.955	-2.139	0.221	2.737	1.397	1.474
Meral Spread	1.236		0.662	1.135	3.883	1.104	1.110
Wrestling	-0.885	-0.868		-0.821	-0.701	2.692	3.762
Do-si-do	-0.725	0.027	-0.532		3.538	-0.045	0.025
Retreat	5.336	4.430	-1.916	1.720		1.027	-3.151
Antenna tap	-0.416	-0.083	2.375	2.258	1.296		-1.785
Strike/ Rip	-0.552	3.032	1.966	-3.610	0.659	-3.527	

Fig. 6. Summary of non-random sequential associations among agonistic behavior patterns identified by cell-wise examination of a significant transition matrix (likelihood ratio $\chi^2 = 255.5$; df = 25; $p \leq 0.001$). Freeman-Tukey deviates exceeding 1.533 [Sokal and Rohlf, 1981] indicate transitions occurring more frequently than expected by chance alone and values less than -1.533 indicate transitions found less frequently than expected. Dark and light shading indicate positive and negative associations, respectively. **A** Freeman-Tukey deviates for all cells of the transition matrix. **B** Behavioral sequence diagram summarizing the results of the Freeman-Tukey analysis. Dark lines with arrow heads indicate transitions that occur more often than expected, light lines with round heads mark transitions that were found less often than predicted.

in future attempts to relate the behavior to its underlying neurobiological mechanisms. The analysis does not say that all components of all patterns are invariably present during agonistic interactions. For example, meral spread (fig. 1a) is a complex display utilizing several different motor elements, but it will not be composed of all the elements in every instance.

The success of the methods of analysis utilized here depends on an adequate choice of the quantified elements. Problems can arise when elements are quantified that are mutually exclusive within an observation interval, resulting in an elevated degree of homogeneity (e.g., animals cannot approach and withdraw from an animal at the same time). The severity of this problem can be judged by examining the factor matrix for an abundance of high negative loadings. Similarly, this approach yields diminishing returns if all the quantified variables are elements that necessarily occur together in the same behavior. Neither of these potential sources of error proved a significant problem in our analyses. This study emphasizes the need to take the multivariate nature of aggression into account. To adequately represent agonistic behavior in lobsters, it must be characterized as a multidimensional profile, with axes representing each behavioral pattern identified by the factor analysis.

The results show that agonistic behavior of juvenile American lobsters is a highly structured behavioral system.



	No Agon. Behav.	No Phys. Contact	Restr. Contact	Claw Lock	Strike/ Rip
No Agon. Behav.		5.150	-3.836	-1.881	-2.654
No Phys. Contact	5.272		2.996	-1.242	-0.878
Restr. Contact	-3.779	7.029		2.199	1.084
Claw Lock	-2.547	1.453	-0.309		3.420
Strike/ Rip	-3.084	-1.483	1.574	3.484	

Fig. 7. Summary of non-random sequential associations among intensity levels identified by cell-wise examination of a significant transition matrix (likelihood ratio $\chi^2 = 255.5$; df = 25; $p \leq 0.001$). Freeman-Tukey deviates exceeding 1.533 [Sokal and Rohlf, 1981] indicate transitions occurring more frequently than expected by chance alone and values less than −1.533 indicate transitions found less frequently than expected. Dark and light shading indicate positive and negative associations, respectively. **A** Freeman-Tukey deviates for all cells of the transition matrix. **B** Behavioral sequence diagram summarizing the results of the Freeman-Tukey analysis. Dark lines with arrowheads indicate transitions that occur more often than expected, light lines with round heads mark transitions that were found less often than predicted.

We have demonstrated a probabilistic occurrence of 6 different agonistic patterns, each one beeing displayed in a highly stereotypical fashion. During some encounters the intensity of fighting escalates into short periods of unrestrained combat, which can result in physical damage to an opponent. Many species with dangerous and damaging weapons that can be used in fights among conspecifics, have evolved mechanisms to diminish the risk of injury [Carpenter et al., 1976; Lumsden and Hölldobler, 1983; Davis et al., 1986]. In lobsters, the presence of a highly structured agonistic behavioral sequence which proceeds according to strict rules of conduct, probably represents another example of this.

The results of this study are consistent with the general predictions presented in the context of Game Theory [Parker, 1974; Parker and Rubinstein, 1981; Maynard-Smith, 1982]. In the presence of a prominent asymmetry, for example in size, encounters among juvenile lobsters are quickly resolved. If such asymmetries do not exist, fights can escalate through various levels of intensity and culminate in a brief period of unrestrained combat. During different stages of the interaction, animals may be assessing each others fighting potential. Agonistic encounters usually begin with a visual display, the meral spread. This behavior achieves an increase in the apparent size of an animal through raising the body high on its walking legs and prominently exhibiting its weaponry [Glass and Huntingford, 1988]. Periods of restrained physical combat ('do-si-do', 'wrestling') follow in which combatants receive direct information about each others vigor and stamina in a 'cheat-proof' fashion [Parker, 1974]. If none of these acts lead to withdrawal of a rival, the fight may enter a brief intense stage during which unrestrained use is made of the claws. The escalation of agonistic interactions seen in juvenile

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