ORIGINAL ARTICLE

Ecology and signal structure drive the evolution of synchronous displays*

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Animal synchrony is found in phylogenetically distant animal groups, indicating behavioral adaptations to different selective pressures and in different signaling modalities. A notable example of synchronous display is found in fiddler crabs in that males wave their single enlarged claw during courtship. They present species-specific signals, which are composed of distinctive movement signatures. Given that synchronous waving has been reported for several fiddler crab species, the display pattern could influence the ability of a given species to sufficiently adjust wave timing to allow for synchrony. In this study, we quantified the wave displays of fiddler crabs to predict their synchronous behavior. We combined this information with the group's phylogenetic relationships to trace the evolution of display synchrony in an animal taxon. We found no phylogenetic signal in interspecific variation in predicted wave synchrony, which mirrors the general nonphylogenetic pattern of synchrony across animal taxa. Interestingly, our analyses show that the phenomenon of synchronization stems from the peculiarities of display pattern, mating systems, and the complexity of microhabitats. This is the first study to combine mathematical simulations and phylogenetic comparative methods to reveal how ecological factors and the mechanics of animal signals affect the evolution of the synchronous phenomena.

KEY WORDS: Collective behavior, ecological pressures, signal evolution, signal pattern, sexual selection.

Synchrony is the most elegant form of collective animal behavior; it can be defined as an adjustment of rhythms between individuals, allowing the formation of a highly coordinated group (Sumpter 2010). In animals, this phenomenon is present in distinct forms and found in phylogenetically distant taxonomic groups with particular modes of communication and ecological pressures (Buck 1988). Collective group behavior can be an adaptive response to mislead predators and increase individual survival, as in the case of fish schools (Ioannou et al. 2012), bird flocks (Hamilton 1971), or treefrog calls (Grafe 1999). In courtship, signal synchronization also increases group visibility and the effectiveness of communicating species identity to conspecifics, which can lead to competition between groups (Greenfield et al. 2017). However, this strategy may not be entirely beneficial, given that at close range, synchrony can mask individual signals and disrupt individual mate quality assessments (Greenfield 1994). In this case, the choosing sex prefers the leader of the synchronous group, given that it sends the most contrasting stimulus (Greenfield and Roizen 1993; Reaney et al. 2008). In these systems, synchrony (an overlapping or alternating coordinated rhythm) is in reality a by-product of the high competition between individuals to signal

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first and avoid being unnoticed (Greenfield et al. 1997). This is a special case of collective animal behavior in which synchrony is an epiphenomenon of behavioral interactions among signalers seeking precedence.

Knowledge of the central drivers of collective behavior, combined with the increase of advanced analytical tools, are essential starting points to expand our perspective of collective systems at a macroevolutionary scale. Although many studies have been identifying key ecological factors promoting the emergence of synchrony (e.g., Greenfield et al. 2017), no study to date has yet traced the evolutionary history of animal synchrony in a broad array of species. To investigate how collective behaviors evolved, we must, therefore, select organisms that offer simplified behavioral ecologies (Sumpter 2010).

Fiddler crabs (Crustacea: Ocypodidae) are a remarkable model system that offers an unparalleled opportunity to explore the selective pressures shaping signal mechanics and allowing for synchronous patterns. These animals live in large mixed-sex and mixed-species colonies where social communications play a central role in survival and reproduction. Among their most characteristic social behaviors, males wave their sexually dimorphic enlarged claw in standardized motions in front and above the body (Crane 1975). The group has a clear behavioral diversification, where each species has its own stereotyped (species-specific) wave display signature, characterized by their distinctive movements (Supporting Information video S1) (How et al. 2009; Perez et al. 2012). Interestingly, in some species, males compete among themselves to give leading waves. Synchrony is nonintentional, as females select for wave display leadership (or perfect alternation) and do not preferably approach synchronous versus nonsynchronous groups (Backwell 2018).

The evolution of fiddler crab wave displays is likely driven by a multitude of factors that influence communication performance. For instance, signal effectiveness is central to animal communication and the elaboration of motion signals is intrinsically connected to the environment in which they are produced (Ramos and Peters 2017). Fiddler crabs are common in intertidal estuaries of subtropical and tropical zones. Their particular sensitivities to environmental conditions make them highly territorial with low population mobility (deRivera et al. 2003). Thus, species distributions are constrained by the mosaic of microhabitats ranging in complexity from open mudflats to close mangroves (Masunari 2006). Species living in specific microhabitats face distinct visual landscapes ultimately posing distinct selective forces in signal broadcasting efficiency (Zeil and Hemmi 2006). Fiddler crabs are active during low tide and rely on visual signals during social interactions (Pope 2005). Their compound eyes are excellent motion detectors, adapted to perceive fine temporal aspects of visual signals. Remarkably, their visual system is also adapted to the environment's topography. Generally, species in open habitats

(mudflats) have high visual depth and detect conspecific signals from long distances. Such visual acuity is not present in species living in mangroves, a likely evolutionary outcome of a low functionality in areas surrounded by roots, rocks, and other obstacles that block their visual field (Zeil et al. 1986). Given these visual landscapes, microhabitats have the potential to affect the evolution signal structure and synchronous signals emergence.

The mating strategy employed by a given species is another important selective force that acts on the evolution of the wave display structure (How et al. 2009). Synchrony is believed to emerge from female preference for leading signals via the perceptual bias for contrasting stimulus, and sexual selection potentially plays a strong role in the evolution of this collective behavior (Backwell 2018). In the species where synchronous waving was confirmed, females leave their own territories during the mating season and search for potential mates among the population of waving males (Backwell 2018). Fiddler crab species with this type of mating system (often called the "female search") incubate their clutches in the safety of the male's burrow (Christy and Salmon 1984). The burrow is therefore an important mating resource that males advertise through wave displays (Crane 1975; Perez et al. 2016). Females with this mating system generally show preference for particular elements of the male wave displays (Oliveira and Custodio 1998; Murai and Backwell 2006; Perez and Backwell 2017). Moreover, wave displays are also under intrasexual selection, as they serve as warning signals to repel intruders. As a consequence, in this system the role of sexual selection is strong and shapes the elaboration of the displays (deRivera and Vehrencamp 2001; How et al. 2009). In the alternative mating system (called the "male search"), females do not require the male's burrow for incubation (Pope 2005). Females therefore do not leave their territories and males are the ones to wander in search for mates. Males are selected based on their persistence, sometimes forcing copulation. Wave displays are mainly directed at short distance range to other males as aggressive signals, but also, although not necessarily, used for courtship preceding mating (Christy and Salmon 1984; deRivera and Vehrencamp 2001; Pope 2005).

The rich interspecific wave display variability, specific habitat preferences, distinct levels of sexual selection, and the evidence of synchrony in some species make fiddler crabs a perfect model system to explore the relationships between ecology, signal structure, and the evolution of animal synchrony. To date, synchrony has been confirmed in 5 among 106 species of fiddler crabs (Backwell 2018). This number is likely to be an underestimate, given that testing each species would involve extensive field studies. In this study, we used the species-specific wave display patterns to predict synchronous behavior of a representative sample of fiddler crab species. We assessed synchronization by running computer simulations in which independently recorded males were set to wave together and compete with each other for leadership. In these

simulations, we first examined whether competition for leadership is in fact the essential element driving synchrony. This method also identified potentially synchronous species and traced the evolution of predicted synchrony in the group. We completed our investigation by using comparative phylogenetic methods to reveal the relationships between predicted synchrony, wave display patterns, mating systems, and the complexity of microhabitats. This is one of the pioneering studies to explore the evolutionary processes that favored synchrony emergence as species adjusted to varying ecologies.

Material and Methods

SAMPLING

We sampled fiddler crabs by visiting three hotspots of species diversity (Australia, Brazil, and Panama, Fig. S1) from November 2010 to February 2017. We gathered a representative sample of species-specific wave display patterns by filming 32 species (four from which are confirmed to present natural synchronous waving), which belong to two of three subfamilies and seven of eleven genera of the currently recognized species (Table S1) (Shih et al. 2016).

CAPTURING SPECIES-SPECIFIC WAVE DISPLAYS

We captured the wave displays by filming active males under natural conditions in the field. We did not experimentally manipulate social context given that a certain social situation might not be an equivalent stimulus for all species. We ensured, however, that the chosen focal animal was engaged in high-intensity wave displays, and that naturally occurring females and males were in their vicinity. We recorded 10 wave displays of five males of each species using a video camera (Panasonic-SDR-H40, JVC-GZ-EX355BAA, Panasonic and JVC, respectively). We focused on the vertical claw path during the wave movement because it is the most visible element to conspecifics (Land and Layne 1995; Zeil and Al-Mutairi 1996). The path can be understood as a signature that the claw draws in the air during the display. To best capture this image, we placed the camera on a tripod (90 cm) directly above the crab and a mirror (40 × 20 cm) on the ground next to his burrow in a 50° angle (Fig. 1A). We waited for the crab to emerge from the burrow and wave, and filmed his reflection on the mirror giving the horizontal perspective of the animal to the camera (Fig. 1B).

Our judgement of what constitutes a wave display followed previous descriptions in the literature (Crane, 1957, 1975; von Hagen 1993). A wave display is defined as a single period of the standardized, rhythmic movement of the enlarged claw while the crab stands on his legs in elevated body posture and is usually separated by clear intervals when the claw is on resting low position (Fig. 1C) (Crane, 1957, 1975; von Hagen 1993). Some species present well-defined single motions (e.g., *Austruca*

perplexa), others present a single movement with stationary moments during the display (e.g., *Leptuca deichmanni*) or a series of successive elevations forming one wave display (e.g., *Tubuca seismella*) (Supporting Information video S1). Additionally, some species can present more than one type of wave display (Perez and Backwell 2017). We focused on the most common type displayed at high intensity (Crane 1975).

VIDEO DIGITIZATION

We digitized the videos frame-by-frame at a rate of 30 frames per second (sufficiently adequate for wave display characterization; Perez et al. 2012) using the software *digilite* created in MATLAB (The MathWorks, Inc., Natick, MA, USA) by Hemmi and Parker. The software allowed for marking the position of the claw tip and carapace tip on the corresponding side of the enlarged claw in each frame. We then tracked the complete series of positions of the major claw in relation to its reference on the carapace tip during the display. Thus, a wave display is given by a series of coordinates of the vertical variation (y-axis) of the claw tip over time (frames), where the claw starts from and ends in a resting position. The result is a claw path or signature (Fig. 1C). It is important to notice that wave displays can vary in duration, which affects the number of frames in the video and, consequently, the number of coordinates they contain. The speed of the claw in specific moments of the display is the main factor that will shape the wave display pattern (Fig. 1C). Finally, in a few cases, the crabs waved in a slight inclination from the ground. We recorded and corrected these inclinations by using rotation matrices (Fig. 1D) (Monteiro and Reis 1999).

NORMALIZATIONS

We normalized the wave displays to facilitate comparison among species. This method normalizes the duration and amplitude of wave displays and preserves the shape and the dynamics of accelerations within each display. We first normalized the duration by identifying the longest wave display among all wave displays of all species. Then by imposing linear interpolation on the original data, we built new coordinates, ensuring that each wave display of each species resulted in the same number of coordinates (n_{max}). Following, we normalized the amplitude by setting the highest and lowest position of a wave display as 1 and –1, respectively, and normalizing the remaining positions between these values (more details in Supporting Information and Fig. S2).

By normalizing the wave displays, we focused solely on the species-specific shape and acceleration patterns. On this note, we highlight that the interspecific pattern is the most consistent and stereotyped element of the waving behavior (Crane 1975; Jordao, Curto, Oliveira 2007; Perez and Backwell 2017). Moreover, although we were careful to film highly active crabs, male quality or social and environmental effects could alter wave display duration
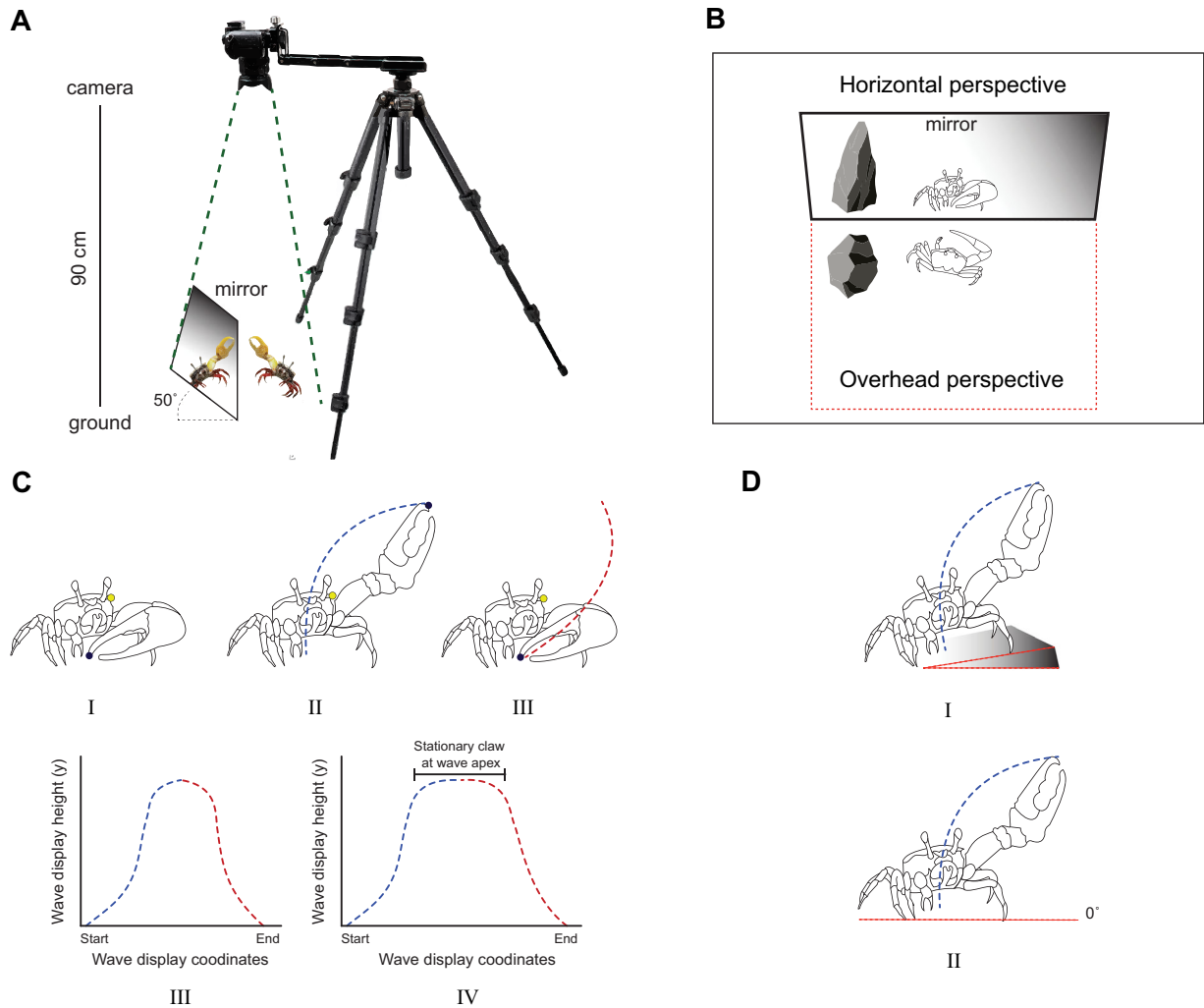
Figure 1. Schematic representations of the experimental setup: (A) camera positioned 90 cm above the crab and a mirror at a 50° angle from the ground; (B) the recorded image is composed of two viewing perspectives, horizontal and overhead, of the crab and objects in its territory (red dashed line). We disregarded images when the crab was facing away from the mirror and the claw path could not be seen. The digitalization processes: (C) digitization in three moments of a fiddler crab wave display: (I) resting position, (II) highest claw position, and (III) back to resting position. The claw displacement through frames is represented by the blue and red dashed lines (black point trajectory), whereas the yellow point on the carapace tip sets a reference for the claw displacement in relation to the body. The vertical claw displacement (display height, y) through time (frames) gives a series of coordinates that compose the wave display (III). The shape of the wave display pattern varies according to the speed of the claw in specific moments of the display, such as a stationary wave peak (IV); (D) a trajectory of a fiddler crab waving represented by the dashed blue line when the animal is (I) on an inclined substrate (indicated by the dashed red lines) and (II) on a flat substrate after the rotation process.

and height (Doherty 1982). Thus, the normalizations minimized the effects of intraspecific heterogeneity (individuality) allowing us to capture the effective interspecific pattern. A wave display is composed by the vertical positions in time and space of each coordinate of its entire normalized series (Fig. S2B).

WAVE DISPLAYS QUANTIFICATION

We quantified wave display patterns by using the method of recurrence quantification. Recurrence analysis is a well-recognized tool to perform visual analysis of embedded information and

patterns in time series (Marwan et al. 2007) and a modern visualization method known as the recurrence plot (RP), was introduced by Eckmann et al. (1987). The RP is a 2D visual tool to identify recurrence in a trajectory phase space, defined as a binary relation:

$$\mathbf{R}_{ij} = \begin{cases} 1 & \text{if } \left\| \overline{y}_i - \overline{y}_j \right\| \le \varepsilon, \\ 0 & \text{if } \left\| \overline{y}_i - \overline{y}_j \right\| > \varepsilon \end{cases}, \qquad (1)$$

where $\| \cdot \|$ is an Euclidian norm, ε is the vicinity threshold parameter. So the RP is a matrix of "ones" and "zeros," denoting two

recurrent and nonrecurrent coordinates (i, j) in a *d-dimensional* phase space, respectively. For each species, we grouped all normalized 10 wave displays of all five individuals totaling $N = 50\, n_{max}$ coordinates. The entire series of wave displays is divided into smaller data-windows of size $K << N$. K is set to be 5–9 n_{max} coordinates. For each interval, windowed computed recurrence quantifiers are captured and the window is moved one coordinate forward.

Based on the information of the RP, it is convenient to define recurrence quantifiers (Zibilut and Webber 1992) to summarize properties of the time series. The primary quantifier is the recurrence rate (RR) that gives us the density of recurrence coordinates of an individual's series of wave displays of the RP. Here we set RR $= 5\%$ (± 2) allowing ε to vary from data-window to data-window. By setting the value of RR, we used two other quantifiers: diagonal length (L) and determinism (DET). L is a measure of the average length of sequential coordinates located in diagonal lines, whereas DET measures the density of diagonal lines in the RP (more details in Supporting Information). In other words, DET quantifies how predictable the wave displays are based on their overall pattern, whereas L is a measure of the average length of predictable periods (sequence of coordinates that are predictable). More predictable wave displays have lower variation, which could lead to higher overall predictability (DET) and longer predictable periods (L). However, these quantifiers do not necessarily correlate. High overall wave display predictability (DET) can be associated with many small predictable periods of the wave display (small L), or few long periods (large L) or even a combination of both. In overall, the level of variability in the wave displays within species plays the main role in generating these results.

COMPETITION FOR LEADERSHIP

We set a condition for competition for leadership by formulating a mathematical model that used the wave displays of individuals in a given species. Unlike Araujo et al. (2013), our model uses the real temporal series and disregards the spatial position of these individuals in relation to one another. We ran the simulations for each pairwise combination between the five individuals filmed, for a total of 10 combinations per species. Each individual started at a random coordinate of its normalized wave display series. An individual is a leader if its coordinate is ahead of the other individual. We promoted the competition for leadership by assuming that the delayed individual is able to double its speed to catch up or surpass the leader. This means that an individual whose coordinate is i will go to coordinate $i + 2$ (under competition). However, the increase in speed only happens when individuals are in the same direction of movement of the wave display (either both in the upward or in the downward movement). Alternatively, when individuals are in the same exact moment of

their wave displays or in alternation (one on the upward and the other on the downward movement), we assumed that the competition for leadership is nullified and the coordinate i maintains its speed $i + 1$ (nullified competition) (more details in Supporting Information).

As individuals may constantly surpass each other, they often change between strategies (under competition and nullified competition). This means that they will never wave in average two times faster than when out of the conditions of the simulation. In fact, the average wave display duration of the individuals in the simulation was 1.04 (*Minuca galapagensis*) to 1.36 (*L. leptodactyla*) times faster than the average duration out of the simulation. These values are supported by empirical evidence on the synchronous species *A. mjoebergi* (individuals wave 1.188 times faster in the presence of males and a female), which matches the speed for this species in the simulation (1.186) (more details in Supporting Information and Fig. S3). Most importantly, it is only possible for individuals to jump to next coordinate $(i + 2)$ and not, for example, half that distance $(i + 1.5)$. In conclusion, to double the speed represents the condition of minimal effort for an individual while any higher speed would favor the synchronization at the expense of greater energy expenditure. Thus, the model obtains a prediction of synchronization under a minimal effort scenario.

The flowchart (Fig. 2) represents the series of decisions taken by two individuals during their interaction. Each simulation is iterated until the individuals reach 20 wave displays combined, which corresponds, on average, 10 wave displays for each individual (the length of a filmed series). To verify the likelihood of synchrony emerging by chance, each simulation was run with and without the condition of competition for leadership. We ran 10,000 simulations for each species grouping them in 10 samples. Each sample corresponds to 100 changes over initial coordinates and wave display sequence for each pair of individuals (among 10 pairwise combinations between individuals). The output of the simulation is exemplified in Figure S4.

MEASURING SYNCHRONY

We predicted synchrony in each simulation by recording the moments of wave displays peaks (value of $y = 1$) of each individual and the temporal delay between individuals. For each wave display peak of individual A that occurred between two consecutive peaks of individual B, we calculated the ratio of the temporal delay of A and the wave display period of B. This ratio was normalized to range from $-180°$ to $180°$, which represents a phase angle (for calculation details see Rorato et al. 2017). The peaks of wave displays are perfectly aligned at $0°$ and completely alternated at $\pm 180°$. The angles were recorded for each simulation giving a complete series of phase angles, which were tested for uniformity using Rayleigh's test (Zar 1999). Higher levels of
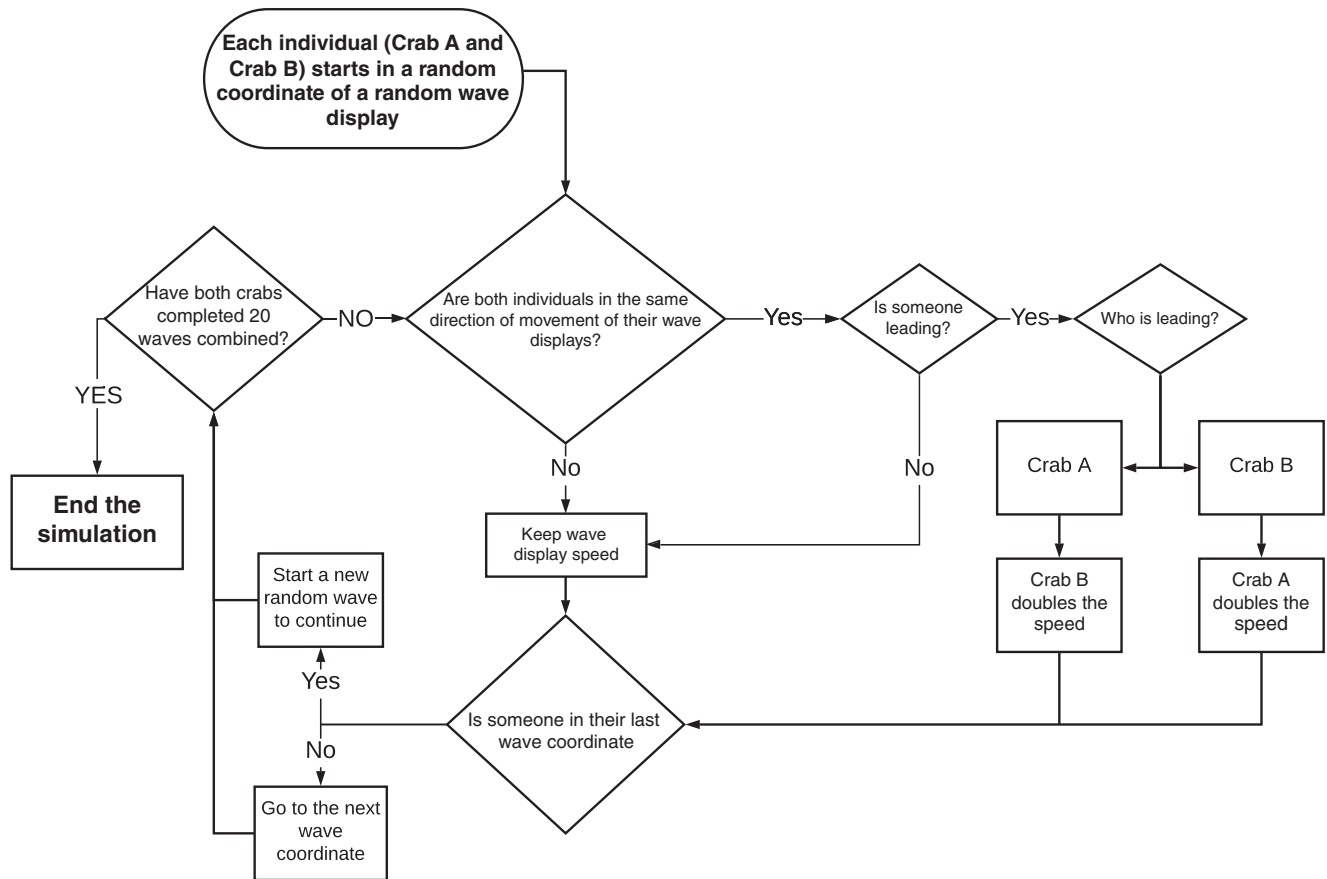
Figure 2. Flowchart of the logical process of the computer simulations.

synchrony are said to occur when the angles are not homogeneously distributed, but lie around a particular angle. Synchrony could be achieved regardless of the phase angle between crabs provided they are recurrent, although our model favored an alignment of the wave displays around 0° (Fig. S5). This also means that the entire series of wave display coordinates of two individuals tend to align (Fig. S4A). We labeled a pair of individuals as being synchronous if the Rayleigh's test gave a $P < 0.05$. Finally, we obtained the percentage of simulations (10 samples, each one with 100 repetitions of 10 pairwise combinations between individuals) that each species synchronized their wave displays as a value for that species to perform synchronous behavior (hereafter called "predicted synchrony," PS).

We highlight that unless PS = 0, a species can, to a certain extent, present synchrony. In addition, although empirical and simulated data on wave display speed in *A. mjoebergi* match (Supporting Information), the values of PS are optimized. This is because wave displays were normalized to obtain the intraspecific wave patterns (minimized environment, social, and individual effects). Thus, the PS values may not exactly match the natural values. However, the crucial aspect about PS values is that they rank the species in relation to one another, ultimately allowing us

to compare species and answer our evolutionary and ecological questions.

MATING TACTIC AND MICROHABITAT

We recorded the predominant mating tactic and microhabitat of each species in the field and complemented this information with an extensive literature review (Table S1). Mating tactic was computed as a categorical variable with two levels namely male search and female search. Some species might adopt both mating strategies, which usually occurs when searching costs shift throughout the mating season, making it beneficial for females to search during some times of the mating season, and to stay in their own territories during other times (Koga et al. 1998; deRivera et al. 2003). However, in these cases, females primarily search and male waving behavior is still highly directed to courtship (deRivera and Vehrencamp 2001). Next, we quantified the complexity of microhabitat of each species by measuring the area occupied by visual barriers in a crab's territory. We considered the main source of visual barriers to be tall and solid obstacles that would block the receiver's visibility of a waving male (e.g., mangrove roots, mud structures, stones, and shells) (Fig. 1B). We carefully identified these obstacles in our videos by looking at the two perspectives of

our experimental setup (overhead and horizontal). We then measured the male's carapace width from the overhead perspective to serve as a scale. Following, we determined the total crab territory as an area that could be occupied by a synchronous group of at least five waving males (approximately 14 by 14 the crab's carapace width). Finally, we used these references to calculate the proportion of area occupied by visual barriers in relation to the crab's territory, where 0 = no barriers and 100 = completely occupied by barriers (Fig. 1B; more details in Supporting Information). We made all measurements using ImageJ software (National Institutes of Health, Bethesda, MD).

PHYLOGENY

Sequences from the studied species were obtained directly from GenBank, and the corresponding accession numbers are indicated in Table S1. Loci were aligned separately using MUSCLE (Edgar 2004) under default parameters, as implemented in EMBL-EBI (McWilliam et al. 2013). After the best model of evolution was determined for each locus in jModeltest 2.1.7 (Darriba et al. 2012) to be GTR+Γ, we ran mixed model Bayesian analysis in BEAST version2.4.5 for 50,000,000 generations, sampling every 50,000th generation, and a relaxed lognormal clock. This ensured average standard deviation of split frequencies were $\ll$0.01 and effective sample sizes of all estimated parameters were $\gg$200. We rooted the tree with the *Uca* clade based on the topology obtained by Shih et al. (2016) (Fig. S6; details in Supporting Information).

STATISTICAL ANALYSES

To assess whether wave display pattern (DET, L) and PS correspond to the species phylogenetic relations, we used a method developed by (Pagel 1999) using the packages phytools (Revell 2012) and picante (Kembel et al. 2010) in R 3.3.2 (R Core Team 2016). It estimates the parameter λ to indicate how well the relatedness between species indicated by the phylogeny will predict similarities of the measured trait. It assumes that the trait evolution follows a Brownian motion or random walk exactly proportional to branch lengths in the phylogeny resulting in a parameter of $\lambda = 1$. Under no phylogenetic signal and other modes of evolution that do not follow the phylogeny, the parameter gives lower values λ. We tested whether the best estimate of λ was significantly greater than 0 using likelihood ratio tests.

To reveal whether PS could be explained by fiddler crab ecology on a macroevolutionary scale, we used phylogenetic generalized least-squares analyses (PGLS) implemented in Geiger (Harmon et al. 2008). The method accounts for the nonindependence of the data due to shared phylogenetic ancestry between species (Martins and Hansen 1997), indicated by the maximum-likelihood estimation of Pagel's coefficient of relatedness λ (Pagel 1999). We predicted the PS by fitting a global model where the explanatory variables were mating tactic, microhabitat, overall wave display predictability (DET) and average length of predictable periods (L), and the interaction between the last two variables. Following, we performed automated model selection in MuMIn (Bartoń 2013) to identify the model containing the strongest power (Burnham and Anderson 2002). The most robust model was selected using delta Akaike Information Criterion, AICc < 2 and weight (Table S2). Model collinearity tests using olsrr package (Hebbali 2018) showed low collinearity in the selected model (all variables TIF < 1.1). Microhabitat, DET, and L were log10 transformed to optimize the models' residual fit. Due to missing information on mating tactic and on GenBank, we excluded *L. batuenta, L. inaequalis, L. oerstedi, L. saltitanta, M. herradurensis, T. hirsutimanus,* and *U. intermedia* from these analyses (Table S1).

ETHICAL NOTE

All procedures were in accordance with the ethical standards of the Australian National University (A2015/42). Filming males caused minimal disturbance.

Results

SPECIES DIFFER IN WAVE DISPLAY PATTERN

High overall wave display predictability (high DET) corresponds to species with quasi-stationary patterns (e.g., *Leptuca deichmanni*). Curiously, these species also generally present short predictable periods (low L) (Fig. S7). Low overall wave display predictability (low DET) is associated to species that wave in successive elevations (e.g., *T. seismella*), which also present relatively short predictable periods (low L). Finally, longer predictable periods (high L) correspond only to species with smooth single sine-wave patterns (e.g., *A. mjoebergi*).

PREDICTED SYNCHRONY VARIES AMONG SPECIES

Our simulations generated a wide range of values of PS among the studies species (from 0.06 to 0.9; Fig. 3, Table S1). Some species showed very low levels of PS (e.g., *Gelasimus vomeris* = 0.06, *T. polita* = 0.06, and *T. seismella* = 0.16), whereas species known to synchronize under natural conditions showed high levels of PS (e.g., *A. perplexa* = 0.9 and *L. leptodactyla* = 0.87), which validates our methods. Curiously, some relatively high values were also found in species where synchrony was never investigated under natural conditions (e.g., *L. inaequalis* = 0.84 and *T. hirsutimanus* = 0.90).

WAVE DISPLAY PATTERN (DET, L) AND PREDICTED SYNCHRONY PRESENT LOW PHYLOGENETIC SIGNAL

The expected variance of PS and wave display pattern (DET, L) do not follow the evolution indicated in the phylogeny (PS, $\lambda < 0.0001$, $P = 1$; logL, $\lambda = 0.0925$, $P = 0.663$; logDET,
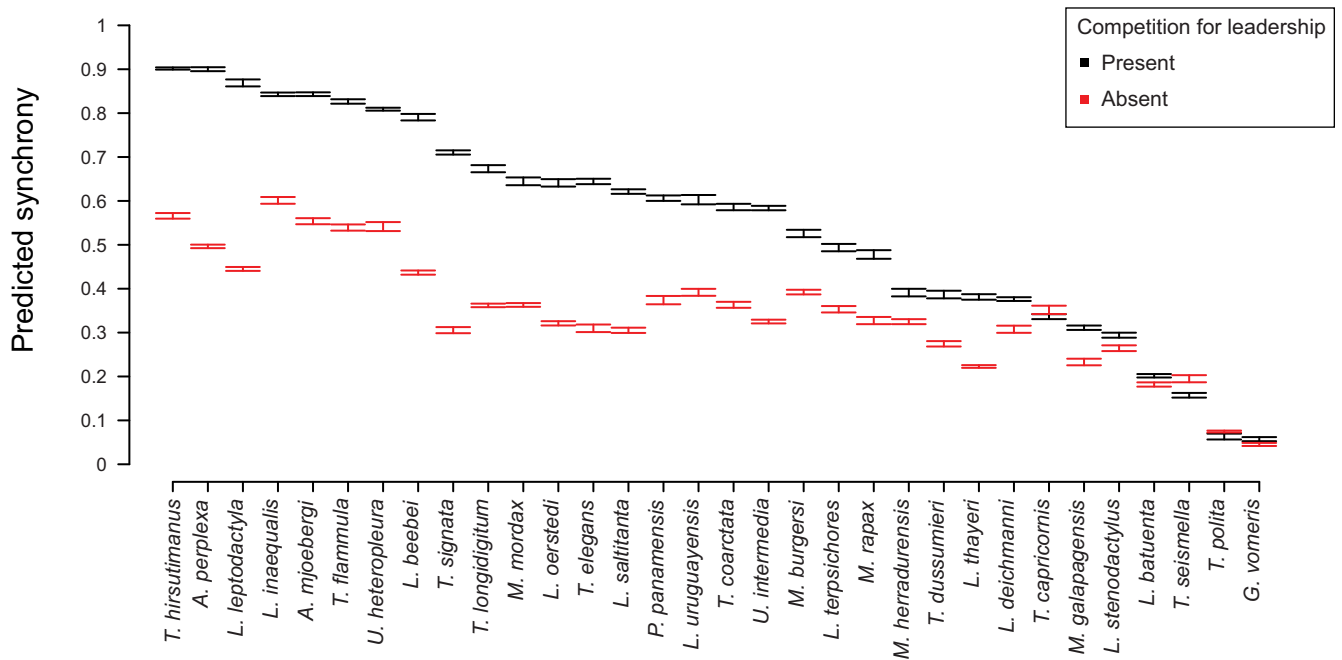
Figure 3. Range between the maximum and minimum predicted synchrony (PS) values obtained from computer simulations and Rayleigh's tests. The PS mean values and their variation are presented for the 32 species and range from 0 (0%) to 1 (100%). The PS values were obtained with (black) and without (red) generating competition for leadership.

$\lambda < 0.0001$, $P = 1$) (Fig. 4). These results suggest that the evolution of wave display pattern and synchrony has not proceeded at a constant rate during the evolution of fiddler crabs, possibly being lost and regained multiple times according to particular evolutionary conditions.

MATING TACTIC, MICROHABITAT, AND WAVE DISPLAY PATTERN INFLUENCE PREDICTED SYNCHRONY

The best model (Table S2) showed that higher PS values are observed in species with female search mating tactic in relation to species with male search mating tactic (PGLS; Female search: $t = 5.63$, SE $= 0.06$, $P < 0.0001$, Fig. 5A). In addition, the level of PS is also higher in species that live in more complex microhabitats (logMicrohabitat: $t = 6.51$, SE $= 0.02$, $P < 0.0001$, Fig. 5B). Finally, more synchronous species have less variable wave patterns and present longer predictable periods (logL: $t = 4.03$, SE $= 0.15$, $P < 0.001$, Fig. 5C). We ran this analysis with 25 of our species due to the lack of information on genetic sequences and/or mating tactic on seven species (Table S1).

Discussion

In the present study, we employ for the first time a combination of mathematical simulations and phylogenetic comparative methods to investigate the evolution of collective animal behavior. Using fiddler crabs as our model system, we show that predicted syn-

chrony (PS) is affected by the species behavioral pattern, mating system, and the visual complexity of microhabitats. We offer an important pioneering step that will guide future and promising research to start building a more comprehensive understanding of the evolutionary mechanisms behind the emergence of one of the most intriguing phenomena in nature.

Effectively measuring the emergence of collective behaviors requires advanced technologies to track, quantify, analyze, and even simulate animal behavior. It is important to note that the lack of observations of synchronous behavior in an organism under natural conditions does not necessarily demonstrate its absence. We circumvented this obstacle by unveiling organisms' probability to present collective behaviors with computer simulations. Using this method, we revealed a wide range of PS values among fiddler crab species (Fig. 3, Table S1). We observed that species that present synchrony under natural conditions were among the ones with the highest PS values, namely *A. mjoebergi, A. perplexa, L. leptodactyla*, and *L. saltitanta*. The close match between the speed reached in our simulations and empirical data in *A. mjoebergi* (details in Supporting Information) also indicates that although our method assumes simplifications, it brings reliability.

However, we also spotted a few incongruences between PS values and field observations, which may result from uncertainties of our simulations. The lowest PS value among confirmed synchronous species (*A. saltitanta)* was of 0.62 and a few high-PS species do not have records of synchronous behavior in the wild. Unlike in other taxa, synchrony in fiddler crabs is not easily
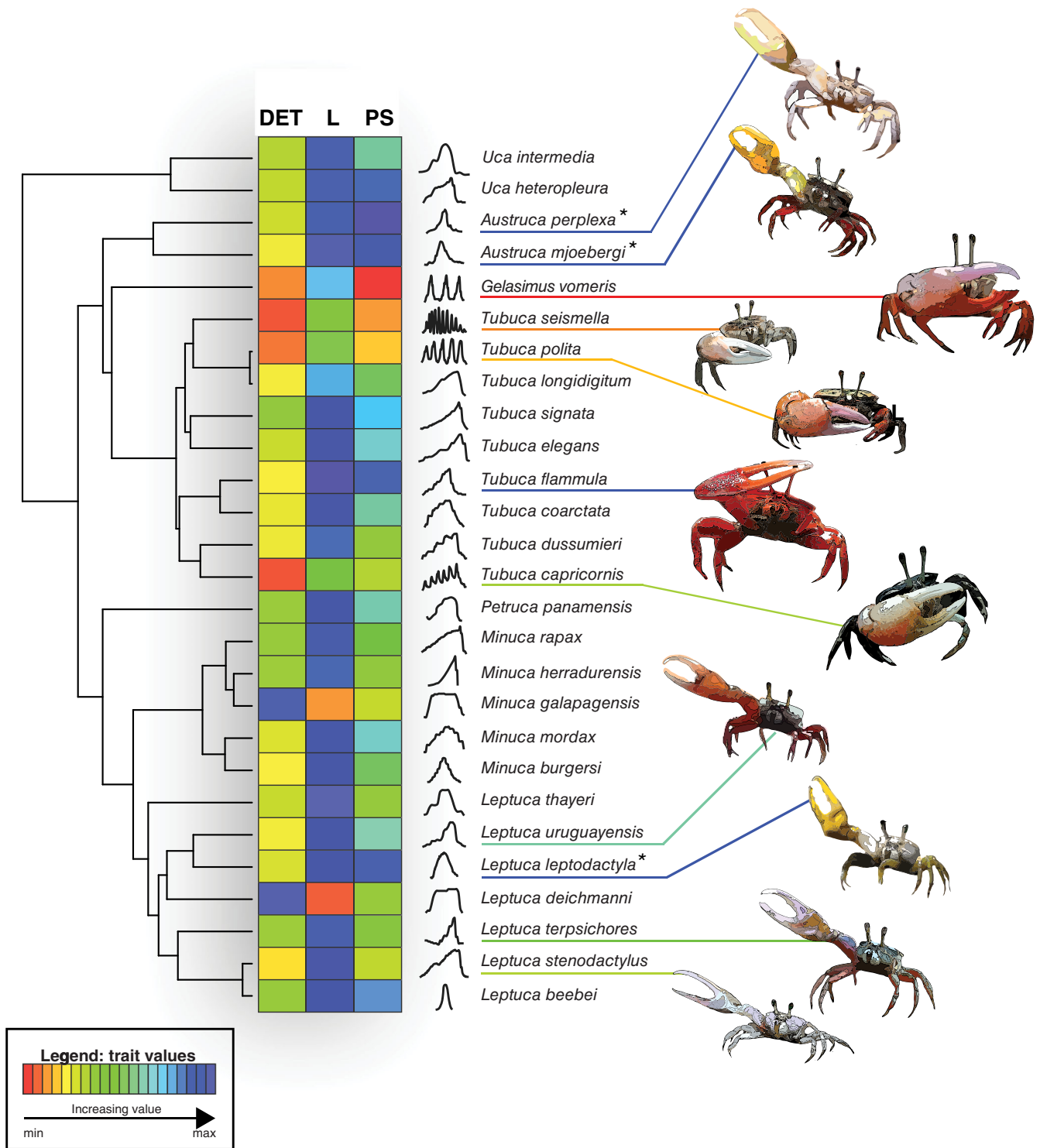
Figure 4. Consensus phylogenetic tree of the 27 fiddler crab species with available gene sequences. Colored boxes alongside phylogeny show from left to right the values for overall wave display predictability (DET), average length of predictable periods (L), and predicted synchrony (PS). Values' intensity is indicated in a color scale from red (lowest values) to blue (highest values). Representative wave display patterns are shown for each species and representative species are indicated by arrows colored according their corresponding PS values. Species with natural synchrony are indicated by *. The relationships among genera are identical to that of Shih et al. (2016). The relationships within genera are largely consistent and the few nodes that disagree were not strongly supported in the phylogeny of Shih et al. (2016) and therefore are likely to simply represent uncertainty in phylogenetic inference.
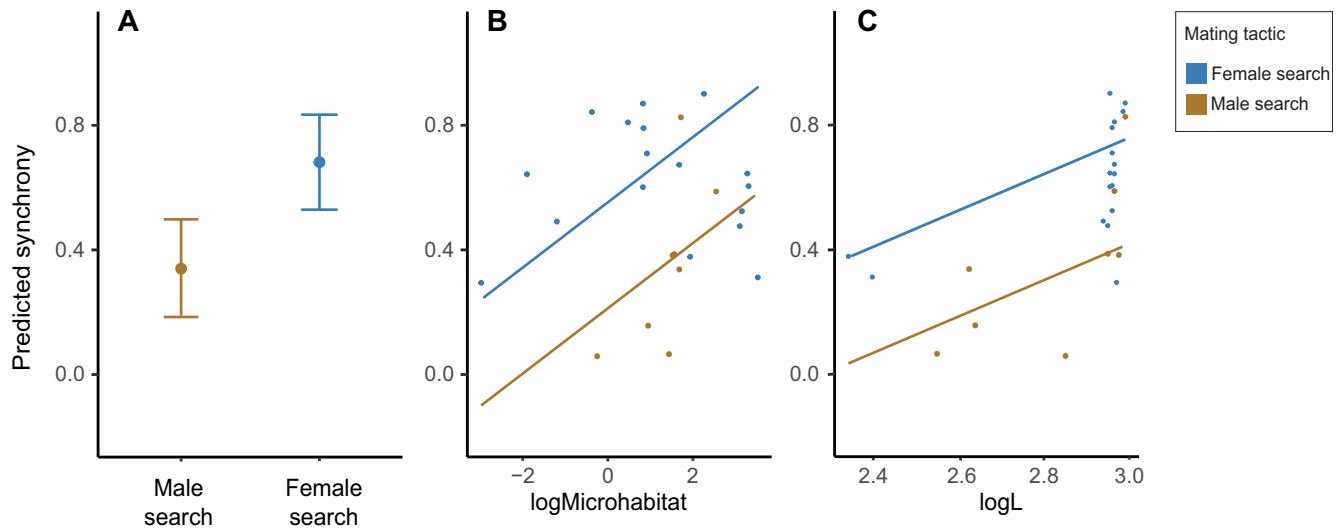
Figure 5. Comparison of predicted synchrony (PS) of 25 fiddler crab species. Plots depict relationships between PS and (A) mating tactic; (B) microhabitat; (C) average length of predictable periods (*L*). The variables microhabitat and *L* are log10 transformed. The trend line was calculated from the phylogenetically corrected model. Each point represents the average value per species.

spotted in the field (Perez personal observations). Signal temporal adjustment is less precise in movement-based communication (Backwell 2018). Higher research effort is necessary to unveil the behavior natural occurrence, as these species are relatively uninvestigated. Alternatively, although some species could express synchronous courtship, they might not compete for leadership under natural conditions. It could be, in fact, counteradaptive for two sympatric species to evolve preference for wave display leadership. Indeed, none of the naturally synchronous species are sympatric (Fig. S1). Nevertheless, the found PS values could be tested in experiments using virtual reality (Stowers et al. 2017) by stimulating the waving behavior of an individual with a cluster of virtual males.

Synchronous courtship is believed to be mostly a direct epiphenomenon of the competition for leadership (Greenfield 1994), which is used as key to explore a wide range of biological systems like anurans (Grafe 1999), fireflies (Buck 1988), and fiddler crabs (Backwell 2018). Yet, we unveil that the competition for leadership is not the sole element responsible for synchrony emergence in our simulations (Fig. 3). This, combined with low phylogenetic signal, suggests that the existence of other factors drive the evolution of courtship synchrony (Greenfield et al. 1997). In this study, we reveal three of these important elements.

First, one of the central goals of understanding collective emergence is tracing the relationships with the mechanisms of animal signaling and movement. Fiddler crab signals are particularly exceptional, as they are the only known case of synchronous courtship composed by body movement (Backwell 2018). Thus, the behavior entails higher elaboration than simplistic acoustical and luminous signals in other synchronous taxa (Buck 1988;

Greenfield and Roizen 1993). In animals, synchrony arises when patterns are similar to each other (predictable), but not necessarily less elaborate. However, more elaborate patterns can be more susceptible to variation, which in turn may compromise predictability and synchrony (Araujo et al. 2013). We show that the characteristic of signal pattern directly influences synchronous systems, as more elaborate wave display patterns had shorter predictable periods (low L), which negatively affected synchronous emergence in our simulations (Fig. 5C). Furthermore, signal pattern cannot be predicted from phylogenetic relationships, which also refutes a traditional assumption that elaborate behaviors are associated to evolutionary derived clades (Sturmbauer et al. 1996; Terhune 2019).

Second, our results support that sexual selection drives the evolution of synchronous courtship in animals. This result strongly corroborates with previous findings that female preference and male signal timing coevolved in isolated populations of katydids (Greenfield et al. 2016). Fiddler crab wave displays are visual stimuli that induce the receiver's alertness and beacon signaler location (Perez et al. 2016). In a group of signaling males, the leading stimulus is most strongly perceived (Reaney et al. 2008). In species with stronger sexual selection, female attraction is essential to mating success (Pope 2005). Thus, there is elevated competition between males, which in turn can lead to synchronous rhythms (Backwell 2018). However, the extent to which female selection promotes the males' ability to synchronize is unclear. We know that sexual selection is strong enough to have shaped male morphology (Bywater et al. 2018). The competition to attract mates could have possibly also shaped wave display pattern in a way that favored synchronous emergence in our simulations.

Finally, we revealed a general positive relationship between PS and complex microhabitats. Fiddler crab eyes are adapted to detecting vertical motions (Zeil et al. 1986; Land and Layne 1995). This perception and visual depth are less accurate in crabs from noisier habitats (Zeil and Hemmi 2006). In more complex visual backgrounds, visual obstacles block the female's visual field, lowering the chances of noticing a candidate. Thus, the competition for leadership must have been favored by the necessity for perception of a signaling individual. More elaborated animal signals have been traditionally associated with more complex habitat structures, as they are more effective beacons of animal presence (Ramos and Peters 2017). Given this panorama, our results show the opposite effect: high PS species with more predictable signal patterns are common from more complex microhabitats. Thus, in courtship synchronous systems, efficient animal perception occurs through leadership, which in turn is achieved with simpler and predictable motion signals. Advanced methods of quantifying the visual topography of microhabitats as well as considering color contrasts with background are important future additions to thoroughly reveal the influence of environment in the evolution of these visual signals.

In this study, we point to significant factors driving the evolution of courtship synchrony. However, we must also recognize that a few other ecological elements not included here can be exciting future additions to build upon the knowledge we offer. First, proximity is important for synchrony emergence (Backwell 2018). Thus, population density is a potential factor that should be explored when data become available. Second, individual condition and social context can affect wave displays (Doherty 1982). We confidently minimized these effects by sampling highly active males, performing wave display normalizations and allowing for display acceleration. However, exploring individuality in synchronous behavior could reveal important patterns. Third, in other taxa, signalers may decelerate or skip a cycle to achieve synchrony or leadership. Although these are valid strategies, future empirical studies are still needed to reveal if fiddler crabs employ them.

The widespread recorded cases of collective behavior in nature indicate their multiple evolutionary origins and points to strong influence from adaptation to particularities of an organism's biology and ecology (Parrish and Edelstein-Keshet 1999; Greenfield et al. 2016). We confirm this hypothesis by revealing that in fiddler crabs closely related species do not necessarily share similar behavioral traits. The low phylogenetic signal in fiddler crab PS is in fact accompanied by a set of evolutionary pressures relating to courtship success and the intertidal life. In summary, we show the importance to account for the relationship between signal elaboration, female selection and perception, and the environment composition when giving an evolutionary look into this remarkable behavior.

We encourage further research to adopt evolutionary approaches to other taxa and expand our understanding of how collective systems evolved. Naturally, each study system has a set of peculiarities that constrain the investigation. Thus, the methods employed here might not be directly applicable across collective systems. Nevertheless, the recent advent of new analytical tools has compelled increasing advances in collective behavior research. In fact, previous findings revealed that group composition and animal individuality in fish schools affect individual performance (Jolles et al. 2017). Moreover, species of fish that are easily adaptable to the movement of other individuals show to be capable to synchronize with heterospecifics, which led to the successful invasion in new environments (Ali et al. 2018). This unveils a valuable starting point to investigate the evolution of collective movement. Different species may present distinct levels of collective group cohesion due to distinct intensities of selective pressures, such as predation (Ioannou et al. 2012).

DATA ARCHIVING
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Supporting Information

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

Fig. S1. Sampling locations in three hotspots of fiddler crab species diversity: Australia, Brazil, and Panama.

Fig. S2. Series of wave displays of an individual of *Leptuca leptodactyla* (A) before normalizations and (B) after temporal and amplitude normalizations where the number of coordinates is equal between displays and wave display height (vertical variation) is equivalent between displays (ranging from –1 to 1).

Fig. S3. Increase in speed under competition for leadership resulted from the theoretical model.

Fig. S4. Series of wave display heights (vertical variation) of two *Leptuca leptodactyla* individuals indicated by red and black (A) with and (B) without the conditions of the simulation where individuals compete for leadership.

Fig. S5. Phase angles between two conservative wave display peaks of two individuals obtained from the theoretical model.

Fig. S6. Tree of the studied species belonging to family Ocypodidae, based on the combined 28S, 16S, and COI markers.

Fig. S7. Quantification of the wave display pattern of 32 species of fiddler crabs using the method of recurrence plot.

Fig. S8. *Austruca mjoebergi* wave display duration in the absence and presence of conspecifics (neighboring males and a female).

Fig. S9. The proportion of area occupied by visual barriers in the territory of the 32 studied species of fiddler crabs.

Fig. S10. Carapace width (mm) of 31 studied species of fiddler crabs.

Table S1. List of species included in the present study with the proportion of species examined in each subgenera indicated by bracketed numbers, followed by: Accession 28S, 16S, and COI numbers; values of predicted synchrony capacity (PS) generated from simulations where individuals of each species are set to wave in a virtual scenario; predominant microhabitat, measured as a proportion of visual barriers in a crab's territory and sampled size indicated in brackets; mating tactics classified in two levels (M) males search for mates where levels of sexual selection are lower (F) females search for mates where levels of sexual selection are higher; measure of average length of predictable periods (L); and measure of overall wave display predictability (DET).

Table S2. Model selection for synchrony capacity in relation to habitat interference (logH), average length of predictable periods (logL), overall wave display predictability (logDET), and mating tactics (M).

Video S1.