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Spatio-Temporal Characteristics of the Prolonged Courtship in Brilliant-Thighed Poison Frogs, *Allobates femoralis*

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ABSTRACT: Prolonged and complex courtship behaviors, involving tactile, acoustic, and visual signals, are common in Neotropical poison frogs (Dendrobatidae). Courtship is an important precursor to mating, but courtship components vary across species. In Brilliant-Thighed Poison Frogs (*Allobates femoralis* [Boulenger 1883]), males guide females to oviposition sites in a so-called “courtship march.” The courtship duration in *A. femoralis* is among the longest known in poison frogs. To gain insights into the functions of courtship, we observed 29 courtship events in an *A. femoralis* population in French Guiana. In addition, we observed multiple courtships of 7 males to assess intra- and interindividual variations in courtship behavior. We recorded temporal, spatial, and behavioral characteristics of courtship and searched for previously deposited clutches in the males' territories. Courtships started in the afternoon and ended on the following morning. During courtship, pairs moved an average of 19 m within an area of about 6 m². Twenty-seven out of 29 courtships (93.1%) resulted in successful oviposition, indicating that females rarely reject males once engaged in courtship. Contrary to previous studies of *A. femoralis*, the spatial and temporal extent of the courtship march did not correlate with the size of a male's territory. Our results indicate that females do not evaluate male quality during courtship but might need an extended courtship phase to verify territory ownership of the courting male and to stimulate ovulation. The prolonged courtship might also be beneficial for spatial learning by females, allowing them to find clutches again in cases of mate loss. Temporal and spatial characteristics vary considerably within and among individuals, and males do not use the same routes in consecutive courtships. However, they probably show females previous clutches. Several courtship traits in our study population differ from *A. femoralis* courtship previously described from Peru and Brazil, indicating that dendrobatid courtship is variable among populations.

Key words: Anurans; Behavior; Mate choice; Minimum convex polygon; Territoriality; Voronoi tessellation

COURTSHIP can be defined as the reproductive communication system preceding a mating event and includes all interactions between a male and a female that lead to pair forming and mating (Bastock 1967; Baylis 1976). Courtship serves multiple purposes such as: (1) confirming the species, sex, and readiness to mate of the potential mating partner (Talyn and Dowse 2004; Saarikettu et al. 2005); (2) evaluating the fitness state of a potential mate (Knapp and Kovach 1991; Sullivan and Kwiatkowski 2007); (3) increasing the success in the competition for mates (Andersson 1994; Rosenthal 2017); (4) avoiding antagonistic interactions between the potential mates (Tinbergen 1960; Barlow 1970); (5) stimulating the reproductive physiology of the potential mating partner (Immonen and Ritchie 2012); or (6) synchronizing the release of the gametes (Amorim et al. 2003).

Courtship is common in many animals; males and females use a broad range of cues to evaluate the mating partner's quality during courtship. In amphibians, complex courtship is more common in terrestrial breeders and species with prolonged breeding seasons (Wells 2007). In species with a short breeding period, female choice is limited because many males compete for few females and, therefore, complex courtship displays have rarely evolved. In contrast, prolonged breeders reproduce over longer periods, promoting the evolution of mate selectivity and complex courtship displays (Wells 2007). In temperate regions, complex

courtship involving visual, tactile, and chemical cues is more common and has been well-studied in urodeles (Halliday 1990; reviewed in Houck and Verrell 1993; Houck and Arnold 2003). Tropical environments have also favored prolonged breeding and courtship in several families of anurans such as Leptodactylidae (Guerra and Ron 2008), Eleutherodactylidae (Townsend and Stewart 1986), Dicroglossidae (Emerson 1992), Centrolenidae (Greer and Wells 1980), Hylidae (Schwartz and Wells 1985), or Dendrobatidae (Summers 1992). Several studies have highlighted the particularly long courtship in Neotropical poison frogs (Dendrobatidae; Wells 1978; Summers 1992; Roithmair 1994a; Costa and Facure 2006; Montanarin et al. 2011; Grant et al. 2017; Souza et al. 2017).

In many poison frogs, males and/or females occupy multi-purpose territories which are used for feeding, advertising, mating, and might also contain access to other essential reproductive resources such as water bodies for tadpoles (Pröhl 2005). Males often call to advertise their territory with the purpose of repelling conspecific male competitors and attracting females. Typically, females initiate courtship by approaching a calling male (Wells 1978; Greer and Wells 1980; Limerick 1980; Bourne et al. 2001) and then following him across the forest floor to an oviposition site (*Dendrobates auratus* [Wells 1978]; *D. tinctorius* [Rojas and Pašukonis 2019]; *Oophaga pumilio* [Limerick 1980; Pröhl and Hödl 1999]; *D. leucomelas* and *O. histrionica* [Summers 1992]; *Ameerega trivittata* and *Allobates femoralis* [Roithmair 1994a]; *Anomaloglossus beebei* [Bourne et al. 2001]; *Al.*

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[=*Colostethus*] *caeruleodactylus* [Lima et al. 2002]; *Am. flavopicta* [Costa and Facure 2006]; *Am. braccata* [Forti et al. 2013]; *Al. paleovarzensis* [Rocha et al. 2018a]; and *Al. subfolionidificans* [Souza et al. 2017]). Complex courtship interactions with tactile, acoustic, and/or visual components are common and often last longer than 1 h (e.g., *D. leucomelas* and *O. histrionica* [Summers 1992]; *Am. braccata* [Forti et al. 2013]; *Al. subfolionidificans* [Souza et al. 2017]). Courtship duration varies widely between poison frog species, but the functional differences resulting from this variation are barely understood.

In previous studies, the following functions of prolonged courtship in poison frogs have been proposed: (1) Males acquire information about the receptive status of the female (*Colostethus inguinalis* [Wells 1980]); (2) Females assess the males' quality (*Al. femoralis* [Roithmair 1992; Montanarin et al. 2011]; *Al. paleovarzensis* [Rocha et al. 2018b]); (3) Females prevent males from mating with other females (*D. auratus* [Summers 1989]); (4) Courtship stimulates ovulation (*An. stepheni* [Juncá 1998]; *An. beebei* [Bourne et al. 2001]). Our study aimed to collect comprehensive data, including intraindividual variation on the courtship behavior of *Al. femoralis* from French Guiana, and compare these findings to previously published data from other populations and to gain insight into possible functions of courtship in our study species.

Allobates femoralis (Dendrobatidae: Aromobatinae; Pyron and Wiens 2011; AmphibiaWeb 2019; but see Grant et al. 2017 and Guillory et al. 2019) is a small, diurnal, leaf litter frog distributed in the Amazon basin and the Guiana shield (Amézquita et al. 2009, 2017; Ferreira et al. 2018). During the prolonged reproductive season, which coincides with the local rainy season (Gottsberger and Gruber 2004; Montanarin et al. 2011), male *Al. femoralis* are highly territorial. They announce territory occupancy by calling from elevated structures on the forest floor (Weygoldt 1980; Hödl 1983; Amézquita et al. 2006). Females actively approach their mating partners and predominately mate with males that have their territories within 20 m to the female's center of activity (Ringler et al. 2012). Males initially respond to females in their view by producing a so-called courtship call, which considerably differs from the advertisement call (see Fig. 1; Weygoldt 1980). The male frequently produces this call during the entire courtship sequence, during which the female follows the male across the forest floor (hereinafter, the "courtship march" *sensu* Montanarin et al. 2011; see also Weygoldt 1980; Hödl 1983; observations by all authors). Pair formation, courtship, and egg deposition take place inside the male's territory (Roithmair 1992; Montanarin et al. 2011; Ringler et al. 2012). Oviposition sites are typically located inside covered or folded leaves in the leaf litter (Montanarin et al. 2011; S. Stückler, personal observation). Females usually leave the oviposition site soon after mating and thus show no prolonged spatial affiliation to their mating partners (Kaefer et al. 2012; Ringler et al. 2012). Males and females are polygamous and mate multiple times within one breeding season. In captivity, females produce a clutch up to every 8 d, with approximately 20 eggs per clutch. After about 3 wk, the eggs hatch into tadpoles (Weygoldt 1980) which the males then carry on their back to aquatic sites, usually outside their territory, where the tadpoles complete development (Ringler et al. 2013). In the field, males care for

up to 6 clutches at the same time (Ursprung et al. 2011; S. Weinlein, personal observation). In captivity, males have been shown to transport all tadpoles in their territory regardless of the paternity (Ringler et al. 2016a), but to cannibalize clutches when taking over a new territory (Ringler et al. 2017). Females perform compensatory tadpole transport only in cases where the respective fathers are missing (Ringler et al. 2015).

Previous studies reported high phenotypic variation in size, color, and calls, and a pronounced genetic variation in different populations across South America (Amézquita et al. 2009, 2017; Simões et al. 2014) as well as a variation in reproductive traits such as amplexus or tactile interactions (Montanarin et al. 2011). Several studies suggest that *Al. femoralis* is a species complex across South America (Amézquita et al. 2009; Simões et al. 2010; Montanarin et al. 2011; Grant et al. 2017). Different explanations for the functions of the prolonged courtship in *Al. femoralis* have been suggested: Roithmair (1992, 1994a) reported a positive correlation between territory size and male mating success in Peru whereas Ursprung et al. (2011) found that male mating success in French Guiana populations was determined by territory ownership without a relationship to territory size. Montanarin et al. (2011) suggested that females use the prolonged courtship to choose a mating partner whereas Ursprung et al. (2011) suggested that females are not choosy and mate with any territorial male calling within audible proximity. Both Roithmair (1994a) and Montanarin et al. (2011) speculated that the prolonged courtship is important for the physiological processes stimulating ovulation in females.

In the present study, we aimed to: (1) quantify how often courtship leads to mating in order to ascertain evidence that courtship is an important component of female mate choice; (2) compare the spatial and temporal extent of the courtship march, and its overlap with the male territory and oviposition sites, seeking evidence that the courtship march indicates a male's territory size (and a proxy for male quality); (3) compare the spatial and temporal extent of the courtship march within and between individuals to gain insights into the variation of this behavior within populations; and (4) describe and compare the behavioral components of courtship and their temporal sequence between our study population in French Guiana and previous studies conducted in Peru and Brazil.

If female frogs were choosy and used courtship to evaluate male quality, we expected that females would reject males during the courtship, and many courtships would not end with oviposition. Based on the idea that males show females their territory during courtship, we also expected a positive correlation between territory size and the length and/or duration of courtships. Previously deposited clutches could indicate past mating success (Dugatkin 1992; Pruett-Jones 1992), and/or territory ownership (cf. Constantz 1985 for a similar effect in fish), as well as previous decisions on oviposition sites that were beneficial for the fate of the clutch (Switzer 1997). Males could guide females during courtship to other clutches in their territory as indicators of their quality and territorial status and to suggest good oviposition sites to the female. Therefore, we expected to find more clutches along the trajectory of the courtship march than in other areas of the territory.

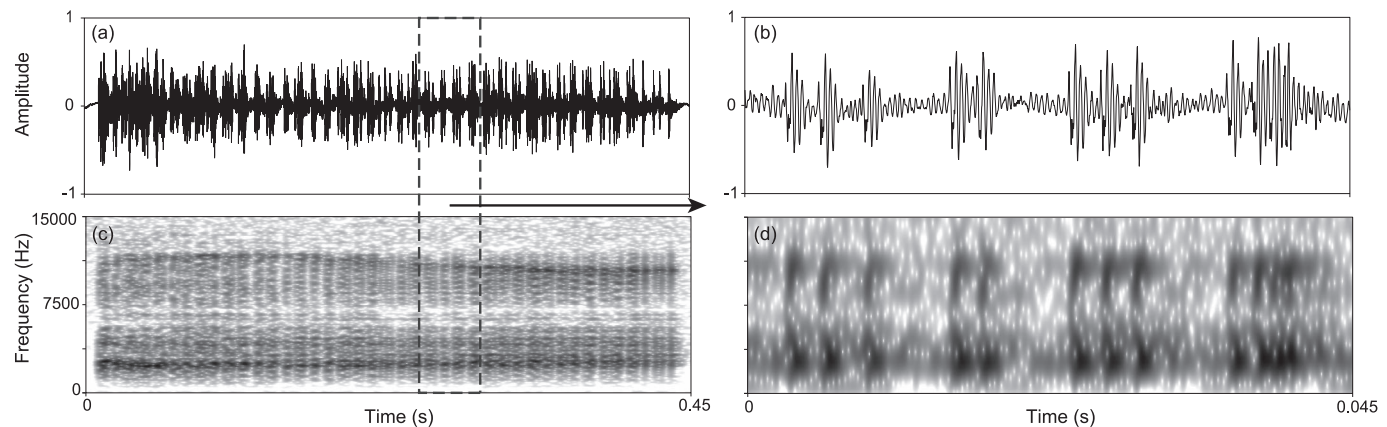


FIG. 1.—Waveforms (a and b) and spectrograms (c and d) showing temporal and spectral characteristics of the *Allobates femoralis* courtship call. (a) and (c) show a full call whereas the portion within the dashed rectangle is enlarged in (b) and (d). The two different scales indicate two levels of amplitude modulation. Spectrograms were generated using a Gaussian FFT window with two different FFT window lengths: (c) 0.01 s and (d) 0.001 s.

MATERIAL AND METHODS

Study Site

We conducted our study in a tropical lowland rainforest next to the field camp ‘Saut Pararé’ (4°02’N, 52°41’W; datum = WGS84) of the French National Center for Scientific Research (CNRS) Nouragues Ecological Research Station within the Nature Reserve “Les Nouragues” in French Guiana (Bongers et al. 2001). The study site was an island of ~5 ha in area in the Arataye River near the field camp (Ringler et al. 2016b). The island is part of a long-term project and there was no *Al. femoralis* population on the island before 2012, when tadpoles were relocated to establish a closed population for research (Ringler et al. 2014). Courtship observations took place during the rainy season from 7 February to 2 April 2016.

In addition to the courtship observations, we conducted a concurrent mark–recapture monitoring of the population during daily surveys from 0900 to 1900 h to estimate the males’ territory sizes. We attempted to sample all males and females and recorded the locations of each frog on a digital map (Ringler et al. 2016b) on a tablet PC (WinTab 8, Odys, Willich, Germany) using the mobile GIS software ArcPad v10.2 (ESRI, Redlands, CA, USA). We sporadically played a conspecific call from a portable loudspeaker (SRS-M30, Sony Corp., Tokyo, Japan; frequency range = 250–20,000 Hz) simulating a territorial intrusion (i.e., conspecific males calling inside the territory) for short time intervals in order to catch male frogs (phonotactic approach behavior; cf. Ursprung et al. 2009), but not during courtship observations. We used the distinct ventral patterns for individual recognition of all frogs (cf. Ringler et al. 2014) using the pattern-matching software Wild-ID v0.4.5 (Bolger et al. 2012).

Behavioral Observations and Mapping

We located courting pairs by actively searching and listening for courtship calls while walking slowly. To obtain a temporally and spatially unbiased sample, we spent approximately equal time per area while surveying the entire island. After finding a courting pair (i.e., a male producing courtship calls and the female following the male), we carefully observed courtship behavior from a distance of 2–3 m while

trying to minimize observer disturbances during courtship activities. We chose the observer distance based on preliminary attempts to observe courtships as a good trade-off between viewing distance in the rainforest understory and minimal disturbance. We distinguished the male from the female based on the inflated vocal sac during calling.

Observations were paused after 1900 h (nighttime) and resumed the following day from 0630 h (daytime) until oviposition took place. As we interrupted the observations only during the frogs’ inactive period from sunset to sunrise, we assumed that we observed the entire sequence of the courtship behavior. At the end of each observation, we caught the male and the female with transparent plastic bags and photographed them for individual identification. We scored courtships ending with a clutch as successful whereas, if one of the frogs stopped courting for more than 60 min and the female–male distance was more than 4 m, we considered the courtship as unsuccessful and we stopped the observation.

For each courtship, we recorded the following parameters: start of observation, end of courtship, time spent during the courtship, time and duration of amplexus, and time when the male and the female left the oviposition site. For spatial analysis, we recorded every position (location and time) where the male stopped for longer than 1–2 min. We did not record movements less than 10 cm. To map the male’s courtship trajectory, we drew a hand sketch of the area (branches, logs, leaves, etc.) and of the frogs’ movements during the observation. Immediately after the courtship, we marked the vertices of the courtship march, based on the sketches with plastic stakes, and measured the distances between consecutive locations with a laser rangefinder (DLE 50, Bosch, Leinfelden-Echterdingen, Germany). For each vertex, we also measured the distance to two reference points (which were either a tree or a reference marker, both shown in the background map), enabling us to then digitize the exact courtship trajectories in AutoCAD 2017 (Autodesk Inc., San Rafael, CA, USA). For the analysis of the spatial data, we then overlaid the courtship trajectories with the background map of the study site (Ringler et al. 2016b) in ArcGIS v10.4 (ESRI).

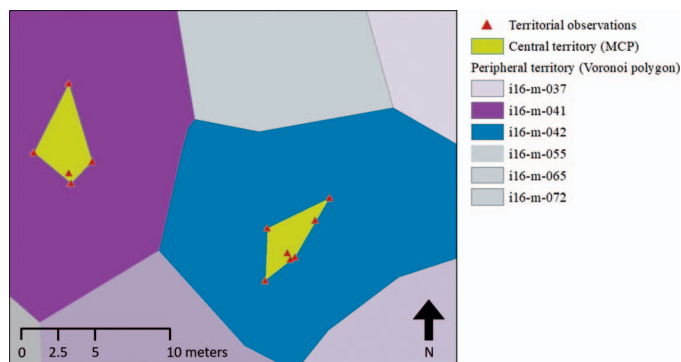


FIG. 2.—Overview map of the central and estimated peripheral territories of two *Allobates femoralis* males and the estimated peripheral territories of neighboring males (gray) in French Guyana. Central territories represent the calling area of *Al. femoralis* males whereas the estimated peripheral territory represents the area that males were expected to defend from conspecific males (cf. Ringler et al. 2011). The yellow polygon represents the central territory, surrounded by the estimated peripheral territory of Male i16-m-041 (purple) and Male i16-m-042 (blue).

Courtship in Relation to Male Territory Size

We calculated central and estimated peripheral territory boundaries of the males based on their locations recorded during the baseline population monitoring. Central territories represented the calling area of *Al. femoralis* males whereas the estimated peripheral territory represented the area which we expect males to defend from conspecific males (cf. Ringler et al. 2011). We used only those males for which we recorded a minimum of five catching points for this analysis and used only those points where the males were observed calling or approaching the simulated call emitted from the loudspeaker.

To estimate the central territory and the peripheral territory, we used two different methods (cf. Ringler et al. 2012; Fig. 2); (1) Minimum convex polygons (MCP) of encounter locations to calculate the central territories, and (2) Voronoi tessellation to estimate peripheral territories. The minimum convex polygon method connects the outer points of a set of points to contain only convex angles ($\leq 180^\circ$ between points). We assumed territory shifts when locations of a male were both further than 10 m away from any other catching point for the same male and recorded more than 10 d apart. In this case, we constructed separate MCPs for each consecutive territory and considered the territory occupied only during the respective courtship observation for our analysis. We excluded five courtship observations from this analysis (CS01, CS03, CS04, CS09, CS15) because we had fewer than five catching points.

To estimate peripheral territories, we used Voronoi tessellation. This method partitions an area into regions based on equidistant midlines between pairs of points (Voronoi 1908). To assure temporal relevance, we only used the previous five (territorial) catching points of each male before the respective courtship observation took place (cf. Tumulty et al. 2018). We excluded two courtship observations (CS01, CS09) because the courtship march was not within the Voronoi polygon, which resulted from too few catching points of the respective males and/or a territory shift immediately before the observed courtship. In two cases, the males had a multi-part Voronoi polygon (CS13,

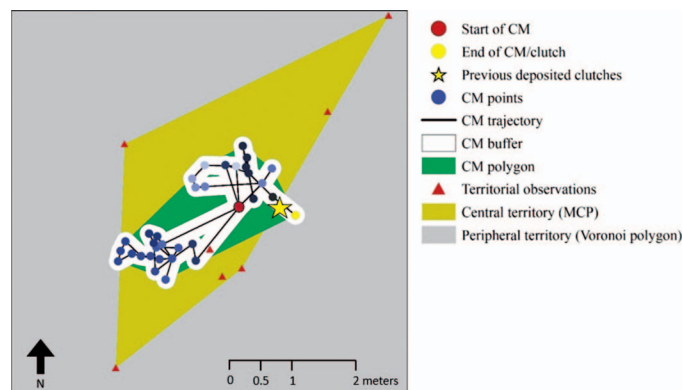


FIG. 3.—A map of an observed courtship march (CM) in French Guyana of the *Allobates femoralis* Male i16-m-042 on 14 March 2016 and the calculated spatial parameters. The red circle indicates the start and the yellow circle indicates the end of the CM, which is also the end of the observation period. The yellow star depicts previously deposited clutches. The blue circles are the recorded CM points, ranging from light blue (start of the observation) to dark blue (end of CM); the black line presents the CM trajectory; the red triangles represent the territorial observation points of the male subject. The central territory of the male is shown in yellow, the peripheral territory in gray. To quantify the area covered during the courtship, we calculated two CM areas: (1) a buffer of 15 cm on both sides of the CM trajectory (white); and (2) minimum convex polygon of the outer CM points (green).

CS15)—for these, we kept only the part that contained/overlapped with the courtship march. Because the shore of the island is steep and not occupied by *Al. femoralis*, we truncated the outer territories midway between a male's outermost locations and the island shore by adding the vertices of the island outline to the points used in the Voronoi tessellation.

To estimate the area that males potentially show to females during courtship, we used two different methods: (1) We buffered the courtship trajectory on each side by 15 cm, termed “courtship buffer,” by using the “Buffer” function in ArcGIS (see Fig. 3). We chose 15 cm along each side because females were not following the male in exactly the same path, and the buffer approximated the area directly explored by the female during courtship. (2) We calculated MCPs from the vertices of the courtship trajectory, termed “courtship polygon” (see Fig. 3). This calculation represented a wider area potentially perceived by females during the courtship. We correlated both estimations of the courtship area with male territory size.

Courtship in Relation to Clutches

To examine whether or not courtship is related to the locations of previously deposited clutches inside a male's territory, we searched for clutches along the courtship trajectory by carefully flipping leaves on the ground for approximately 60 min (depending on the length of the courtship march) after the courtship. Given that females do not follow the male in the exact same path, we considered all encountered clutches within the courtship buffer. As a control, we searched the male's territory outside the courtship buffer for the same duration. We also recorded the locations of all discovered clutches in the digital background map of the island with ArcPad. To evaluate if courtship was related to previously deposited clutches in each male's territory (e.g., if a male shows his previous

clutches to females), we compared the number of clutches found within the courtship buffer to the number found outside.

Within Individual Courtship Variation

We observed 7 of the 15 different males repeatedly during courtship (2 males 4 times, 3 males 3 times, and 2 males 2 times), allowing us to analyze the intraindividual variability of courtship. To investigate if the male frog was guiding (different) females along a similar route in subsequent courtships, we used the ArcGIS function "Intersect" to assess the area/percentage of overlap between the courtship march buffers of that male.

Data Analysis

To test for normality of the variables, we used the Shapiro–Wilk normality test. Variables that significantly deviated from a normal distribution (central territory size, estimated peripheral territory size, duration in the morning, courtship buffer size, and courtship polygon size) were log transformed. To test if the length (distance in meters), the total duration, the duration in the morning, the courtship buffer size, or the courtship polygon size are indicators of male territory size (central territory and/or estimated peripheral territory), we used Pearson correlations. To analyze the variation within and between the male subjects, we calculated coefficients of variation (CV) and compared them with analyses of variance (ANOVA) followed by Tukey post hoc tests. Statistical analyses were conducted in R (v3.4.2; R Core Team 2019).

The sample sizes varied depending on the specific analysis because not all parameters were available for each male frog. For the general description of the courtship, we considered only each male's first courtship ($n = 15$). We calculated Voronoi polygons and MCPs for 10 out of the 15 males, and these males were considered in the correlation of courtship extent (temporal and spatial) to male territory size. The remaining 5 males had too few catching points to calculate reliable territory sizes. For the temporal analysis, we excluded 1 of the 10 males because we could not record some data on account of heavy rainfall during the observation. To relate the courtship to the already present clutches in the territory, we considered 13 out of these 15 males. We excluded 2 males because we had too few points for a reliable calculation of their territory sizes. To analyze the variability within and between the males, we considered 6 of the 7 males, which we observed repeatedly. One male shifted its territory between courtship observations and therefore was excluded from the dataset.

RESULTS

Courtship Success

In total, we observed 29 courtships by 15 different males. We observed 7 of these 15 males multiple times (2–4 times) in different courtships. Twenty-seven out of 29 courtships ended with a successful oviposition whereas 2 courtships were unsuccessful because the female stopped following the male and left the male's territory before oviposition occurred. In one unsuccessful case, the pair was courting in the afternoon, but while the female remained overnight in the male's territory, she did not resume courtship activities

on the next morning. After 86 min of courtship calling in the morning, the male stopped and the female left the male. In the second case, the pair was in courtship for 205 min in the afternoon; the female stayed overnight and resumed courting the next morning for another 157 min. Then, shortly after the neighboring male started calling, the female left the first male and directly approached the calling neighbor. The rejected male switched to advertisement calling after the female left. After 24 min of courtship with the neighboring male, they deposited a clutch inside a leaf, 30 cm next to another recent clutch.

General Description

As soon as a female came close to a male (~ 30 cm), the male typically turned toward the female and alternately emitted advertisement and courtship calls. The courtship calls consist of a broadband burst of pulses produced at a high rate that sounded like a quiet buzz to a human ear (Fig. 1). Each courtship call was approximately 0.5–1 s in duration and 2500–2700 Hz in dominant frequency and sounded different from a tonal advertisement call (see Gasser et al. 2009). The courtship call was softer than the advertisement call and appeared to be used only for short-distance communication between male and female.

As soon as the female jumped toward the male, the male moved slightly away (approximately 10–20 cm) from the female. Then, the male stopped and emitted courtship calls until the female followed. On those occasions when the female did not follow the male within a few minutes, or the female was out of sight, the male switched back to the more prominent advertisement call. This sequence was continued until sunset (between 1845 to 1900 h), when both frogs hid under leaves and interrupted their courtship activities. In 28 out of 29 observations, the female stayed overnight inside the male's territory.

In the morning (between 0630 to 0645 h), the pair resumed their courtship. The male frog always entered the oviposition site first (in the cases where the sequence was observed; $n = 9$). While sitting inside a folded or covered leaf, the male emitted courtship calls until the female joined him. Sometimes the females jumped outside shortly after entering the site and then the pair continued with courtship. In 5 out of 29 cases, the frogs later returned and mated inside a previously visited leaf. In all other cases ($n = 24$), the pair mated in a leaf which they had not previously visited.

Mating behavior.—We observed cephalic amplexus in 15 out of 27 matings, mostly in the early morning ($\bar{X} \pm 1$ SD = 0757 h \pm 68 min; Fig. 4b). In the other 14 cases the pair was hidden, but we could observe both partners crawl under the same leaf and we found a fresh clutch after they had left the nest. At the oviposition site, the pairs initially sat head-to-head while the male emitted courtship calls. Then, the female turned to face away from the male frog. The male climbed on top of the female and grasped her head with his front legs. This cephalic amplexus lasted an average of 2 min and males left the site soon after amplexus ($n = 9$). During and after amplexus, the female laid her eggs on the leaf and then spent an average of 77.4 min ($n = 8$) at the oviposition site before leaving the nest.

In 8 out of 10 males, the central territory was inside the estimated peripheral territory. In the other cases, the central territory was partly outside the peripheral territory. This



FIG. 4.—Mating sequence of *Allobates femoralis* in French Guiana: (a) male inside the oviposition site, female in front of it; (b) male and female in cephalic amplexus; (c) female inside the oviposition site on the freshly laid eggs.

apparent paradox resulted from the distinct approach used to estimate central and peripheral territories. In the spatial and temporal comparisons, we subtracted the central territory from the peripheral territory and treated them separately in the following results.

Temporal pattern.—Courtship observations started in the afternoon (median \pm interquartile range [iqr] = 1715 h \pm 53 min) and ended on the following morning (median \pm iqr = 0811 h \pm 58 min), leading to a total average courtship duration of 15 h (range = 806–1043 min, $n = 15$). When subtracting the 11 nocturnal hours (1900 to 0600 h) from the total duration, courtships lasted an average of 222 min (range = 116–353 min, $n = 15$; Table 1). On average, more than half of the duration of all courtships was spent within the respective male's peripheral territory, about 25% of the time was spent in the central territory, and less than 10% was spent outside the male's estimated territory ($n = 9$, see Fig. 5 and Table 2).

Spatial pattern.—The length of the courtship march ranged from 7.67–41.25 m ($\bar{X} \pm SD = 18.69 \pm 8.77$ m, $n = 15$). The courtship polygons ranged from 1.50–115.52 m² and the courtship buffer from 1.62–11.21 m² ($n = 10$; Table 2). On average, more than half of each courtship march was located in the peripheral territory, around 15% was located in the central territory, and around 20% outside the male's territory ($n = 10$; Table 2). None of the observed ovipositions occurred inside the central territory; 6 clutches were deposited inside the peripheral territory and 3 clutches outside ($n = 9$; Table 2). On average, the courtship buffer overlapped with 0.01% of the male's central territory (range = 0.00%–25.47%) and 0.98% of the peripheral territory (range = 0.06%–3.19%; $n = 10$).

Courtship in Relation to Male Territory Size

The peripheral territories ranged from 121.34 to 1116.30 m² (median \pm iqr = 259 \pm 342.17 m², $n = 13$) and the central territories from 1.39–44.37 m² (median \pm iqr = 11 \pm 6.91 m², $n = 10$; Table 1). The Pearson product-moment correlation model revealed that male territory size (central territory and peripheral territory) was neither predictable by a courtship's path length, the total duration, the duration in the morning, nor the courtship buffer and the courtship polygon.

Courtship in Relation to Clutches

In total, we found six previously deposited clutches inside the courtship buffers of 13 different males. We only found

one clutch outside the courtship buffer but still within the estimated peripheral territory of the male. Observed ovipositions never took place in the central territory: in six cases the pair laid the clutch inside the estimated peripheral territory and in three cases outside the territory. Only nine males were included in this comparison because they were the only frogs with sufficient data points to reliably calculate central and estimated peripheral territories.

Within Individual Courtship Variation

We did not find consistency within single individuals, in either length or duration of their courtships (CVs and ANOVA). As the duration depends on the length of the courtship, we calculated residuals (time~length). We also did not find consistency within the individuals using this metric. The median overlap of the courtship buffer between consecutive courtships by a single male was 0.81%, ranging from 0.24–2.83% ($n = 6$; see two extreme examples in Figs. 6 and 7).

DISCUSSION

In contrast to previous studies of *Al. femoralis* populations, courtships at our study site typically ended in

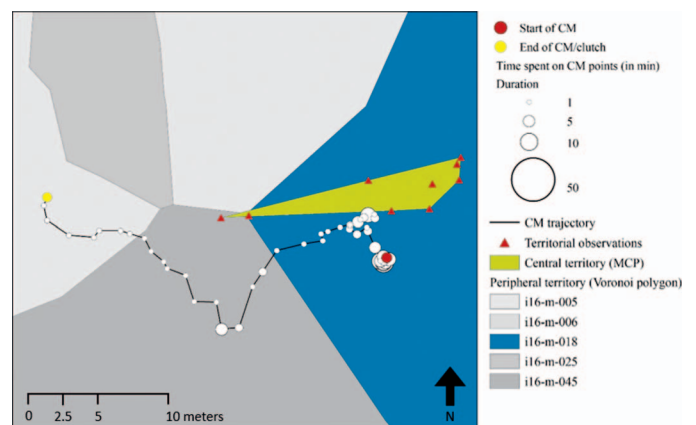


FIG. 5.—A map of an observed courtship march (CM) in French Guiana of the *Allobates femoralis* Male i16-m-018 on 30 March 2016. The red circle represents the start of the CM. The clutch produced after the CM is represented by the yellow circle, which is also the end of the observation period. The size of each circle (white) corresponds to the time (in minutes) the male spent at each location. The CM trajectory is shown as the black line; the red triangles represent the territorial observation points of the male. The central territory (minimum convex polygons) is labeled in blue and surrounded by peripheral territories (different shade of gray).

TABLE 1.—Temporal and spatial patterns of the courtship march (CM) of *Allobates femoralis* in French Guiana.^a

Pattern	\bar{X}	SD	Median	Q1	Q3	IQR	n
Temporal							
Start of CM	1702 h	62 min	1715 h	1645 h	1738 h	53 min	15
End of CM	0818 h	61 min	0811 h	0737 h	0835 h	58 min	15
Total duration (min)	911.5	71.7	907	870	952.5	82.5	15
Duration without night (min)	221.5	71.7	217	180	262.5	82.5	15
Spatial							
Length of CM (m)	18.69	8.77	16.61	12.45	23.17	10.72	15
Area of CM buffer (m ²)	4.36	2.75	3.14	2.63	5.36	2.73	10
Area of CM polygon (m ²)	18.17	33.10	5.79	3.24	10.39	7.15	10
Peripheral territory (m ²)	384.27	312.22	259	166.66	508.83	342.17	13
Central territory (m ²)	13.98	12.53	11	8.04	14.95	6.91	10
CM buffer coverage (%) of the peripheral territory	1.33	1.17	0.98	0.24	2.46	2.22	10
CM buffer coverage (%) of the central territory	4.01	8.04	0.01	0	1.64	1.64	10
CM buffer overlap within individuals (%)	1.05	0.86	0.81	0.45	1.14	0.69	6

^a Q = quartiles (Q1 = first quartile; Q3 = third quartile); IQR = interquartile range.

successful mating (27 out of 29 observations), with only two ending in rejection. Roithmair (1992) observed seven courtships ending with mating compared to 34 ending in rejection, while Montanarin et al. (2011) reported two courtships ending in rejection out of nine observations. These differences could be caused by demographic differences of the studied populations such as population structure (e.g., male–female ratio, population density), seasonal variations within or between reproductive seasons, or be attributable to methodological differences (e.g., effects of the observation protocol). Our observations indicate that most females choose before courtship or indiscriminately mate with a territorial male. In the first case, females could decide whether or not to engage in courtship based on cues such as male calls. In other anuran species, female preferences for specific calling patterns are common (e.g., preference for a high calling rate in one population of *Hyla chrysoscelis* [Gerhardt 2005]; preference for a lower-than-average call frequency in *Engystomops pustulosus* [Ryan et al. 1990] and *Acris crepitans* [Ryan et al. 1992]). In the second case, females did not choose a mate based on male or territory quality but mated with any territorial male to minimize the risk of mating with a low-quality male. Based on genetic data, Ursprung et al.

(2011) suggested that *Al. femoralis* females are not choosy and mate with any male located within 20 m of their resting site. Females of *O. pumilio* were also nonselective and chose the closest calling males (Meuche et al. 2013). Our findings corroborate this pattern, indicating that female *Al. femoralis* from French Guiana do not assess male quality during courtship.

The courtship march in *Al. femoralis* is among the longest previously observed in poison frogs (Table 3). The temporal and spatial extents of courtships were even slightly underestimated because we never observed the exact initiation of the courtship. Our observations before the study, as well as a concurrent telemetry study of females (M.-T. Fischer, personal observation), had already suggested that courtships generally start in the late afternoon, which coincides with the time range of courtship encounters in our study. Therefore, the missing period at the onset of each courtship likely did not have a strong effect on our results. Furthermore, using a parameter independent of the starting time, we correlated the territory size with the courtship duration observed in the morning before mating, which yielded the same outcome.

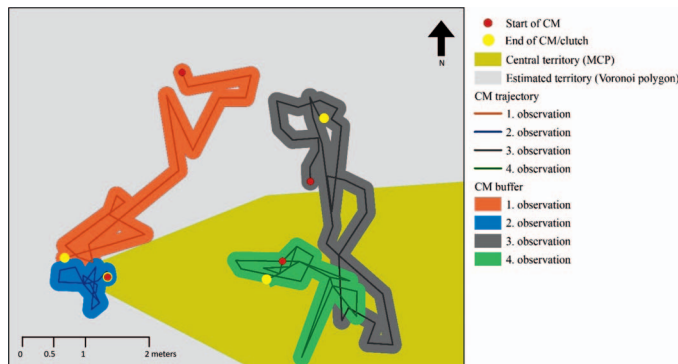


FIG. 6.—Map of four observations of different courtship marches (CM) by the same *Allobates femoralis* (Male i16-m-003), with little overlap between observations. The red circle represents the start of the CM. The clutch produced after the CM is represented by the yellow circle, which is also the end of the courtship observation. The CM trajectory and the CM buffer are shown in different colors. The central territory is in yellow and the estimated peripheral territory is in gray.

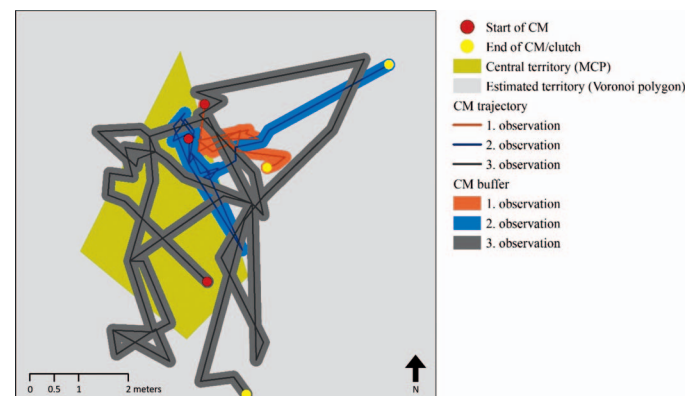


FIG. 7.—Map of three observations of different courtship marches (CM) by the same *Allobates femoralis* (Male i16-m-041) with higher overlap than shown in Fig. 6. The red circle represents the start of the CM. The clutch produced after the CM is represented by the yellow circle, which is also the end of the courtship observation. The trajectory and buffer for each CM are shown in different colors. The central territory is in yellow and the estimated peripheral territory is in gray.

TABLE 2.—Proportions of time spent ($n = 9$) and distance covered ($n = 10$) in different areas during the courtship march of Brilliant-Thighed Frogs (*Allobates femoralis*) in French Guiana. The locations of the oviposition sites are given based on territory parameters for the male frog ($n = 9$).

Area	Time in %	Distance in %	Oviposition
Central territory	23.63	14.67	0
Peripheral territory	67.92	64.59	6
Outside	8.45	20.74	3

Courtship in Relation to Male Territory Size

We did not find any correlation between courtship area and male territory size. This result indicates that *Al. femoralis* females cannot use the courtship interaction to assess the size of a male's territory. We observed that the courtship trajectory, and even oviposition, might occasionally occur outside the male's territory. Oviposition happened very rarely in the center of the territory. Previous studies reported that courtship and oviposition of *Al. femoralis* take place only in the males' territory (Roithmair 1994a; Ringler et al. 2013). In total, 20% of the length ($n = 10$) and ~8% of the time ($n = 9$) of the courtship, and three ovipositions (out of nine courtships observed), occurred outside the male territories (peripheral or central; Table 2). We need to interpret these results with caution because male territories change over time (e.g., change of territory extent, location, or even ownership). It is unlikely that territorial changes and methodological issues account for the large proportion of courtship observed outside the territory, however, because we used two different methods to calculate males' territories. The Voronoi tessellation even takes the time of observation into account and overestimates (rather than underestimating) the size of a territory. Our finding that courtship and oviposition sometimes occur outside the males' territory was unexpected, given that territoriality is a common feature across most dendrobatid frogs and represents an important resource for successful reproduction (Pröhl 2005; Ursprung et al. 2011). In many dendrobatid species, territory characteristics such as size and oviposition sites are important indicators of male quality (Wells 2007). In *Am. trivittata* (Roithmair 1994a), *O. pumilio* (Pröhl and Hödl 1999), and *Al. paleovarzensis* (Rocha et al. 2018b), male mating success was positively correlated with territory size and thus might represent a primary indicator for male quality in these species. Rocha et al. (2018b) suggested that female *Al. paleovarzensis* use courtship to assess the males' territory size because it correlated positively with courtship duration and numbers of male matings. Possibly, *Al. femoralis* females do not assess the size of a male territory during courtship but

rather make sure that the potential mate is the territory owner.

Courtship in Relation to Clutches

In six cases, the courtship trajectory closely passed freshly laid clutches, but we only found one fresh clutch in the control areas. Previous studies in fish showed that females often copy the choice of other females, and males with eggs are preferably chosen by females (Dugatkin 1992; Pruett-Jones 1992). In *Al. femoralis*, females might recognize previously deposited clutches inside the male's territory during courtship—our data show that the trajectories of courtship marches often passed close to clutches from previous matings. Interestingly, experiments in the lab showed that female *Al. femoralis* cannibalize unrelated clutches in the absence of a guarding male (Spring et al. 2019), suggesting that males should avoid passing by previously laid clutches during courtship in order to minimize the risk of cannibalism by females. In these experiments, however, females were kept in close vicinity to the clutches and their motivation to cannibalize unrelated clutches might be different under natural conditions. Further studies are needed to clarify the relation between clutches already present in a male's territory and courtship marches in *Al. femoralis*.

The prolonged courtship behavior in dendrobatids is important to locate a suitable oviposition site (Lima et al. 2002; Montanarin et al. 2011; Forti et al. 2013; Souza et al. 2017; Rocha et al. 2018a). In *Al. femoralis*, oviposition sites are rain-protected leaves in the leaf litter. We observed males going under a leaf, followed by the female, who exited shortly after entering. This sequence was repeated (up to four times) at different locations, until both frogs stayed under a leaf and oviposition took place. This behavior indicates that the female evaluates the oviposition site presented by the male (cf. Switzer 1997). Showing previous clutches to females during courtship might likewise serve to demonstrate high quality, which is a common strategy in fish (e.g., Unger and Sargent 1988). Thus, the prolonged courtship in *Al. femoralis* is likely to serve as a mechanism to locate suitable oviposition sites.

We suggest that female *Al. femoralis* use the courtship sequence to explore and learn the area, especially with relation to the exact location of the clutch. Male *Al. femoralis* are predominantly responsible for tadpole transport, but females perform compensatory tadpole transport in cases where the respective fathers are missing (Ringler et al. 2015). Recent studies have shown that *Al. femoralis* females use indirect spatial cues to discriminate between their own and an unrelated clutch (Ringler et al. 2015, 2016a). From

TABLE 3.—Summary of different courtship studies of seven poison frog species. Courtship durations are reported without the overnight period, where no courtship activity takes place.

Species	Courtship duration (min)	Tadpole-transporting sex	Reference
<i>Allobates femoralis</i>	116–353 ($n = 15$)	Primarily male, facultative female	This study
<i>Allobates paleovarzensis</i>	1–17 ($n = 9$)	Male	Rocha et al. 2018a
<i>Allobates subfolionidificans</i>	17–257 ($n = 24$)	Primarily male, occasionally female	Souza et al. 2017
<i>Ameerega braccata</i>	3–90 ($n = 13$)	Male only	Forti et al. 2013
<i>Ameerega trivittata</i>	12–68 ($n = 17$)	Male only	Roithmair 1994a,b
<i>Dendrobates leucomelas</i>	246 \pm 20 ($n = 8$)	Male only	Summers 1992
<i>Oophaga histrionica</i>	71 \pm 7 ($n = 9$)	Female only	Walls 1994

TABLE 4.—Comparison of the reproductive traits in three different populations of *Allobates femoralis*: (1) Panguana Biological Station, Peru (Roithmair 1992, 1994a); (2) Reserva Florestal Adolpho Ducke, Brazil (Montanarin et al. 2011); and (3) this study. Table modified after Montanarin et al. 2011.

Reproductive trait	Brazil ¹	Peru ²	French Guiana
Advertisement call (no. of notes) ³	4	3	4
Throat display	Present	Not reported	Absent
Limb lifting	Present	Not reported	Absent
Circling	Present	Not reported	Absent
Leg stretching (color display)	Present	Not reported	Absent
Tactile interactions	Present	Absent	Absent
Amplexus	Cephalic	Absent	Cephalic
Mating period (time of amplexus) ⁴	0700–0950 h (<i>n</i> = 7)	0605–0635 h (<i>n</i> = 7)	0657–1057 h (<i>n</i> = 9)
Courtship duration (min)	40–210 (<i>n</i> = 7)	95–225 (<i>n</i> = 7)	32–275 (<i>n</i> = 15)
Male, time remaining at nest (min)	2.6 (1–4, <i>n</i> = 5)	13 (7–18, <i>n</i> = 7)	3.3 (2–8, <i>n</i> = 9)
Female, time remaining at nest (min)	51 (32–60, <i>n</i> = 7)	37.8 (18–60, <i>n</i> = 7)	77.4 (8–269, <i>n</i> = 8)

¹ Montanarin et al. (2011).

² Roithmair (1992, 1994a).

³ According to Amézquita et al. (2009).

⁴ Note the different time zones and time of sunrise: Brazil/Amazonas: GMT-4, 0556 to 0607 h; Peru/Panguana: GMT-5, 0528 to 0613 h; French Guiana/Parare: GMT-3, 0627 to 0643 h.

the male's perspective, locating multiple clutches close together facilitates defending, tending, and recalling their locations whereas females should rather prefer oviposition sites that are not too close to clutches of other females to avoid accidentally transporting unrelated clutches in case they have to perform this task (cf. Ringler et al. 2015, 2016a). Prolonged courtship might help in learning the location of the oviposition site which, in turn, would be useful for subsequent tadpole transport. Future studies should focus on the relationship between female vs. male parental care and courtship duration in different poison frog species.

All observed females stayed overnight in a male's territory before mating, indicating that females might need time to reach the physiological condition for oviposition. As *Al. femoralis* are prolonged opportunistic breeders, and environmental conditions vary, females would benefit from having the ability to control the timing of ovulation and to wait for optimal conditions. When followed in the field, female *Al. femoralis* laid clutches at variable intervals (5–12 d, *n* = 7; M.-T. Fischer, personal observation). Previous studies of other frog species suggest that prolonged courtship is important for stimulating ovulation (e.g., *An. stepheni* [Juncá 1998] and *Al. caeruleodactylus* [Lima et al. 2002]). Roithmair (1994a) and Montanarin et al. (2011) proposed that female *Al. femoralis* need to experience the courtship call and/or behavior to reach the necessary physiological condition for oviposition. In *An. stepheni*, the male amplexes the female two times and each amplexus lasts around 50 min. The first amplexus is without oviposition but after approximately 5 h, the male amplexes the female again and oviposition occurs. Juncá (1998) demonstrated that the first amplexus stimulates ovulation and that the females need the time between the two bouts of amplexus to ovulate. Female *Ranitomeya vanzolinii* had ovulated eggs during courtship, but not when they were without a male (Caldwell and Oliveira 1999). Females in *Al. femoralis* might thus require prolonged courtship to stimulate their physiological condition for ovulation.

Comparison to other Populations across South America

Our results show differences in the reproductive traits compared to other *Al. femoralis* populations across South America (Peru and Brazil). Although some differences across

populations could be attributed to observation protocols and analytic procedures, the overall courtship behavior observed from our study population in French Guiana differed in many aspects from populations in Peru and Brazil. For example, compared to the other populations, we observed that courtship interactions between male and female frogs last longer, that tactile or visual signaling is absent during courtship, and that courtship culminates in cephalic amplexus (see Table 4). The variation of courtship and reproductive behaviors in different *Al. femoralis* populations across South America is ideal to investigate the functions of different courtship components, but more-standardized, methodological approaches to studying poison frog courtship are needed.

CONCLUSIONS

For the *Al. femoralis* population at the Les Nouragues Nature Reserve, our study shows that (1) most courtships are successful, meaning that females rarely reject males during courtship; (2) spatial and temporal characteristics of a courtship are generally not correlated with the males' territory size, but might be necessary to verify territory ownership; (3) spatio-temporal characteristics of courtship behavior vary between and within individuals; and (4) behavioral traits of courtship differ from those of other *Al. femoralis* populations. Based on our findings, we suggest that in *Al. femoralis* courtship is of little importance for mate evaluation in our study population, but serves to corroborate male territory ownership, identify a suitable oviposition site, stimulate the ovulation of the female, and to gather spatial information needed for parental care. Further studies in a more controlled environment, and comparisons between populations and across closely related species, will provide deeper insights into the functions of complex courtship behavior.

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committee of the CNRS Nouragues Ecological Research Station and the institutional ethics board of the University of Vienna (2016-003). We confirm that all animal handling procedures were conducted in strict accordance with current French and European Union law and followed the guidelines of the Association for the Study of Animal Behaviour (ASAB 2018). We are grateful to the staff of CNRS Guyane, especially to P. Gaucher, for logistic support in French Guiana. Thanks to T. Bath for copy editing, C. Rodríguez for statistical advice, and three anonymous reviewers for comments on an earlier draft of this article.

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