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Male hilltopping and territorial behavior of the butterfly *Actinote zikani* (Heliconiinae: Acraeini)

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Abstract
This study has the objective of describing the hilltopping and territorial behavior of adult males of the bivoltine butterfly *Actinote zikani* during six flying seasons (2016-2019). Males used the same areas and these territories presented patterns of horizontal and vertical stratifications during all seasons. The amount of solar radiation on the black wings of *A. zikani* seems to contribute to rapid warming this butterfly, that together with the protection against stronger winds were the main variables that altered the *A. zikani* behavior and consequently shaped the territories. Some males were recorded flying throughout the territory during more than two hours without landing, inspecting agonistically other intruder conspecifics and other insects. Some aerial fights with conspecifics resulted in strifes on the ground when each male used their genitalia to infringe damages to the other. Here, males of *A. zikani*, broke the paradigm that butterflies have no mechanical weapons to use against other individuals. In these territorial areas, even in seasons where few floral resources were available, no individuals of this species were observed foraging on flowers or inflorescences during periods of territorial behavior.

Keywords: Atlantic forest, thermoregulation, agonistic behavior, threatened species

Introduction
The *Actinote zikani* butterfly is known from only one population discovered in 1991 near the Paranapiacaba village [1]. Together with *A. eberti* [2], and its just-described sister species, *A. keithbrowni* [3], they are species that require conservationist attention, especially because they inhabit the Atlantic Forest, a biome that has been heavily impacted by anthropogenic activities. *Actinote zikani* has black wing color pattern that facilitates rapid warm-up under cold conditions, like *A. morio*, which has a wider geographic distribution than *A. zikani* [4] and, as in all known *Actinote* species the individuals of all stages are chemically protected liberating HCN when predated [5].
Males of *Actinote zikani* at the top of the mountain, above the village of Paranapiacaba presents peculiar behaviors and strategies to maintain their territories and defend their encounters with females [6]. Several records of insect swarms on hilltops are known from the late 19th century and throughout the 20th century (see Shields [7] for a detailed study).
In this study we used the term hilltopping to the individuals of both sexes of *A. zikani* butterflies which have an innate behavior of flying to the summit of Morrão hill in Paranapiacaba village (Serra do Mar) to mate (see Shields [7], and Scott [8] for definitions). The term territory is used as "an area occupied more or less exclusively by an animal or group of animals by means of repulsion through overt defense or advertisement" [9].
The objective of this study was to describe the hilltopping and territorial behavior of adult males of the bivoltine butterfly *Actinote zikani* during the 2016 spring season, both flying seasons of 2017 and 2018, and autumn season of 2019. Female behavior, including mating, will be presented in another paper.

Materials and methods
Study area
Detailed description of territories, topography, and climate of the study area is in Francini *et al.* [10].

Weather parameters recording

Microclimate conditions during samplings were done measuring the surface temperatures of vegetation and other substrates, the intensity of solar radiation, the air temperature at shade, the relative humidity, and the surface temperature of white and black polystyrene sponges.

The instantaneous recording of the surface temperatures of vegetation and other substrates was measured with a Hikari HT-450 infrared thermometer with 0.1°C accuracy pointed at 1m from the target. Also, the instantaneous recording of the intensity of solar radiation (in W/m^2) was made using an Instrutherm MES-100 radiometer.

In some behavioral observations, microclimate data was continuously recorded by a portable station formed by digital sensors connected to Arduino systems based on an 8-bit microprocessor Atmega2560 powered by a 5V Power bank battery programmed to collect data every 10 seconds. It measured three types of parameters: (1) air temperature and relative humidity; (2) the surface temperature of black and white polystyrene sponges and (3) the luminous intensity (Lux).

The temperature of black and white of surfaces were recorded using a 5 mm thick sponges with 50 x 50 mm on each side, each one in direct contact with a digital sensor DS18B20 inserted in the center. These sensors were used to simulate the minimum and maximum potential heat absorption from direct solar radiation by the bodies of butterflies as described in other works [11-13].

Behavior recording

At least a twelve-different type of digital cameras were used to for shooting or making videos. All recorded times are local time (Brasília; GMT -3), ignoring the daylight-saving time that started in late spring of each year. For time standardization, the clocks of all equipment were synchronized with an accuracy of 2 minutes.

At the end of study, more than two thousand photographs and dozens of videos were done and analyzed.

Frequency of wing beat was estimated analyzing manually, frame by frame, videos done at 120 fps.

In the spring of 2017, one researcher collected, marked, released, and recaptured all sighted individuals on six dates in a 16-day period, from October 28 to November 12, from the TOWER 2 to TOWER 3, in the Bela Vista Trail. The butterflies were carefully collected with an entomological net and marked with colored enamel spots or numbered using a Sharpie permanent marker pen.

Distances traveled by males in normal patrol flight were estimated using yellow plastic bottle caps placed apart at 5 meters intervals.

Statistical analysis

Data were analyzed using R software v. 4.2.1 [14] with RStudio interface v. 2022.07.1+554 [15] using packages mgcv V. 1.8-40 [16] and ggplot2 v.3.3.6 [17].

Results

Sampling effort

The study was done from October 28, 2016, to November 22, 2019. In 69 fieldtrips we collected data accumulating a total of 197 hours of observation. In 45 samplings (65.2%), at least two researchers were present, working in different points to maximize data.

Fifty-eight samplings (96.7%) were done during flying seasons in Bela Vista trail (Table 1).

Table 1: Synthesis of collected data during the study exclusively in the Bela Vista trail from 2016 to 2019. FSIGHT: date of first sight. LSIGHT: date of last sight.TT: Total of samplings in the season. YY: Samplings with adults present. NN: Samplings without adults present. SE: sampling effort (days); HO: sampling effort (hours); FI: total of recorded fights between males; FA: total of recorded drops to the substrate; MA: number of males; FE: number of females; AL: total recorded; SR: sex-ratio.

Year	Season	Fight	Light	TT	YY	NN	SE	HO	FI	FA	MA	FM	AL	SR
2017	AUTUMN	March 26	April 15	9	7	2	17	51	2	7	27	0	27	1.00:0.00
2017	SPRING	October 8	November 15	14	9	5	18	39	44	4	60	1	61	0.98:0.02
2018	AUTUMN	March 5	March 31	6	5	1	5	20	57	1	15	1	16	0.94:0.06
2018	SPRING	October 23	December 4	10	8	2	10	31	23	3	17	1	18	0.94:0.06
2019	AUTUMN	March 19	April 2	3	2	1	3	11	10	3	14	5	19	0.74:0.26
2019	SPRING	October 18	November 22	3	0	3	3	7	6	2	1	6	7	0.14:0.86
			Totals	45	31	14	60	174	142	20	145	14	159	0.91:0.09

Hilltopping and territorial behavior of males was observed in 60 dates (56.7%), between TOWER 2 and TOWER 3 (Fig. 1 A) in the main territories T1-T2. (Fig. 1 B).

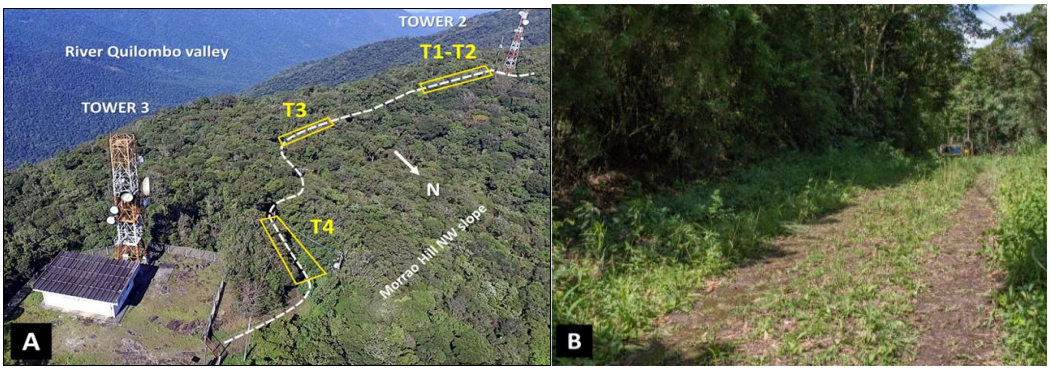


Fig 1: (A) Aerial drone view of the study area showing territories T1-T2 near TOWER 2 where most observations were done. (B) Main territory (T1-T2) viewed from T3 side on November 18, 2018.

The other nine trips were done along the Pontinha Trail to detect the presence of the larval food plant, *Mikania biformis*

DC. There, and along the dirt road to Paranapiacaba-Taquarussu only four adults were recorded in the flight season foraging in the available flowering plants of *Vernonanthura polyanthes* (Sprengel) Vega & Dematteis (Asteraceae) (Fig. 2 A), and *Aegiphila* sp. (Verbenaceae) (Fig. 2 B).
Unfortunately, the COVID19 pandemic create a situation which restricted the fieldwork during 2020-2021.

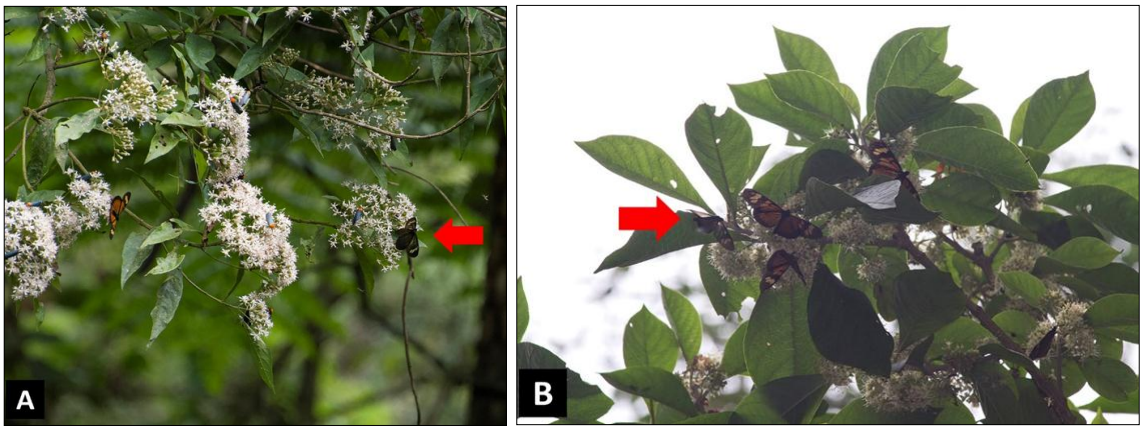


Fig 2: During the study, individuals of *A. zikani* (red arrows) were observed foraging only outside the territories of hilltop (Bela Vista). One record was in the Pontinha trail (A) on November 17, 2016, using *Vernonanthura polyanthes* (Asteraceae), and other in the in the Taquarussu dirt road, using flowers of *Aegiphila* sp. (Verbenaceae) on November 26, 2017. The other individuals are *Actinote parapheles* males.

Male hilltopping and territoriality

Males of *A. zikani* have strong hilltopping behavior and when reaching to hilltop, they start his patrol flight and establish territorial areas in short linear transects.
More than 70% of the first sights (n = 32) showed that the performance began in the lower portion of territory T2 and, according to the temperature at shade, sun path and wind, the male slowly approaches to the end of territory T1, the main territory.
Activities related to feeding or oviposition were not observed in *A. zikani* main territory.
A. zikani males have some particularities as the formation of adjacent linear territories side by side, without the visible presence of satellite males perched in the surroundings. After suddenly appearing and losing fights, the intruders are likely to stay near the main territories to reduce their battle costs and avoid repeated fights they have already lost. Furthermore, these losing males, although occupying "lower quality" areas outside the best position to observe the surrounding area, may still conserve energy and copulate with some passing females. Although the approaches during copulation are like territorial fights, the females do not seem to resist or run away easily unlike the challenging males.

Persistency and resiliency in territory and the use of perches for resting or basking

Five newborn males were recorded in cold days, resting in early morning in territorial areas between TOWER 1 and TOWER 2. In all cases the air temperature at shade was less than 17°C (Fig. 3 C).
During the flight seasons, from 09:00 h to 12:00 h the males begin their territorial activity and use perches only when air temperature at shade is above 17°C, the wind velocity is below 10 m/s, and the sun was not obscured by clouds.
When these conditions are not met, they land on vegetation for basking. These perches have a height ranging between half and two meters (Fig. 2 A, C-D).
The territory-owning male stay perched until it can glide and warm up its body and starts patrolling. Therefore, landing was correlated to thermoregulatory needs and happen when body temperature is lowered by environmental conditions as showed ahead.

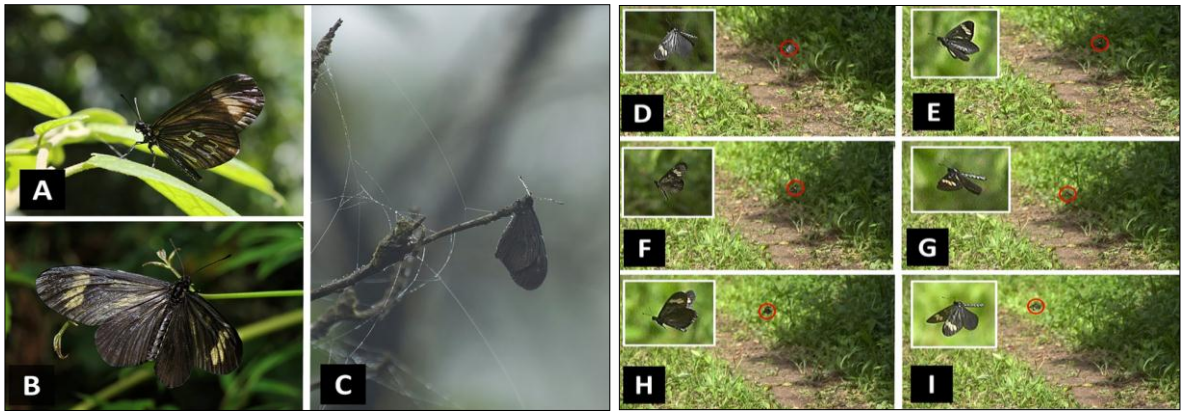


Fig 3: Males of *A. zikani*. (A) Landed in a perch in the main territorial area after been marked with number 53 on November 12, 2017. (B) Basking in a perch in the main territorial area on December 4, 2018. (C) Resting in a cold morning on October 31, 2017. (D-I) A sequence of normal patrolling flight of a males of *A. zikani* recorded for two seconds, showing the different positions of the wings during the flight. Height above ground near 0.5 m in a sunny day without wind and air temperature at shade of 21°C), When flying or landed with open wings they show the dark wing pattern with the yellow spot in forewing.

Flight behavior, aerial chases, and strife in the substrate

Two types of flight were found on males.

When environmental conditions are optimal (temperature, cloud cover and wind), and the male territory-owner finds no intruders in his line of sight he proceeds the normal patrol flight. He flies horizontally a certain distance, opening and closing the wings (Fig. 3 D-I) and, eventually, gliding and, suddenly, doing 180 degrees turns and returning. This cycle can be repeated dozens of times from edge to edge of territory.

When the weather conditions are not optimal the flight is interrupted and the male lands on a substrate (Fig. 3 A-B). During ascending normal flight, the frequency of wing beat varied from 9 to 12 beats/s (n = 17) which can reach 15-20 beats/s in rapid ascending flights in aerial fights (n = 8).

The distances traveled by the normal patrol flight by nine different males, on different dates, and without any landing, was variable (Fig. 4). The maximum distance traveled on the outward and return flight was 30m.

Other flight type happens when the probability of encounter rate with other flying insects or conspecific males is high. In this situation, the flight is more agitated, varying continuously the height above the ground.

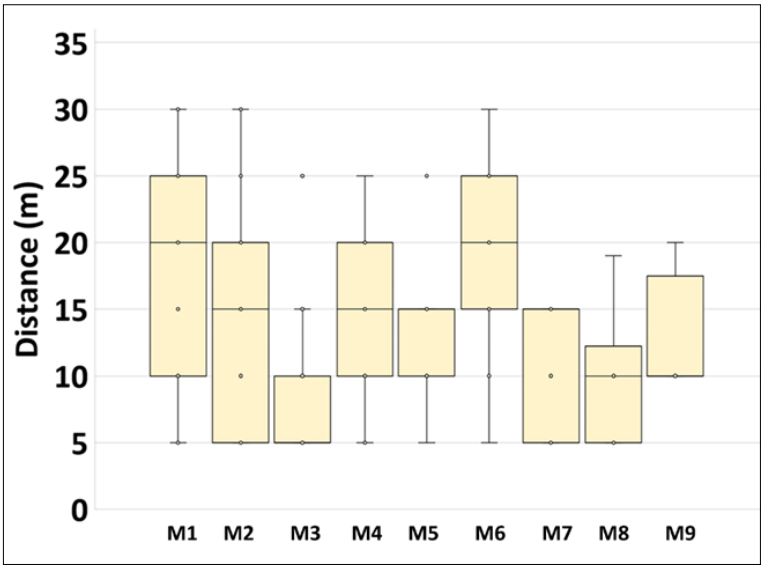


Fig 4: Distances traveled by the normal patrol flight by nine different males (M1 to M9) in territory T1. Temperature at shade during flights varied between 21-22°C (spring season). Data using number of records within each 5 m classes of distance (see methodology). Horizontal line inside boxes is the median.

A typical male territorial behavior was recorded on April 4, 2017, when a single male maintained a territorial flight in territory T4 at TOWER 3, in the end of Bela Vista trail. Its flight varied from 1 to 10 m above ground. From 09:05h to 12:30h it remained in this area flying and landing on some specific perch. From 11:06h to 12:32h, the flight duration was variable and determined mainly by the appearing of other insects. When flying, this male also persecuted several other insects as flies, bees, and other butterflies. Generally, they were quickly expulsed from its territory.

When a flying insect fly above, the landed male quickly responded taking off and flying in the encounter of the invader (Fig. 5 A) and the responses to them appeared not to be correlated with the intruder's size. After the interception, the territory owner usually returns to his perch and continues the surveillance. While the male keeps guard over the territory from a perch, it exhibits a peculiar behavior turning its head around to detect the passing intruders (n = 3) (Fig. 5 B-C).

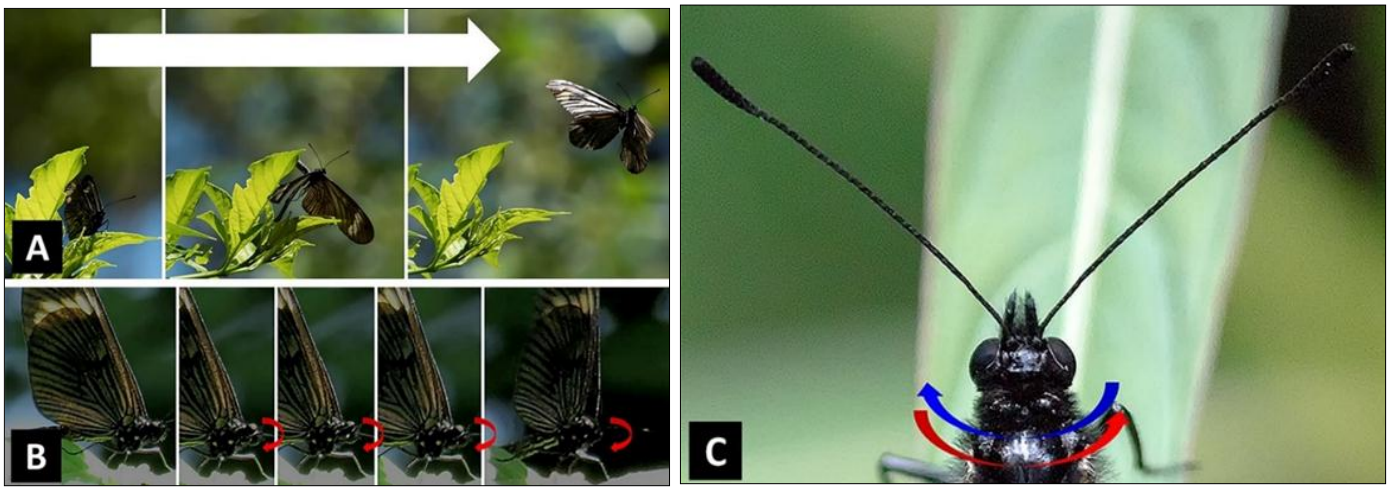


Fig. 5: (A-C) A sequence of a landed male of *A. zikani* which quickly responded to a presence of other intruder male in its territorial area on November 12, 2017. (D) Lateral movement of the male head to accompany an intruder in its territorial area.

When the invader was another *A. zikani* male, the perch owner responded more agonistically (n =9). In this case, the

pair goes up, 10-15m above ground and done a falling spiral flight to ground (Table 1, FI; Fig. 6 A-C). During the descent flight they fight, and their bodies may come in contact (Fig. 6 D).

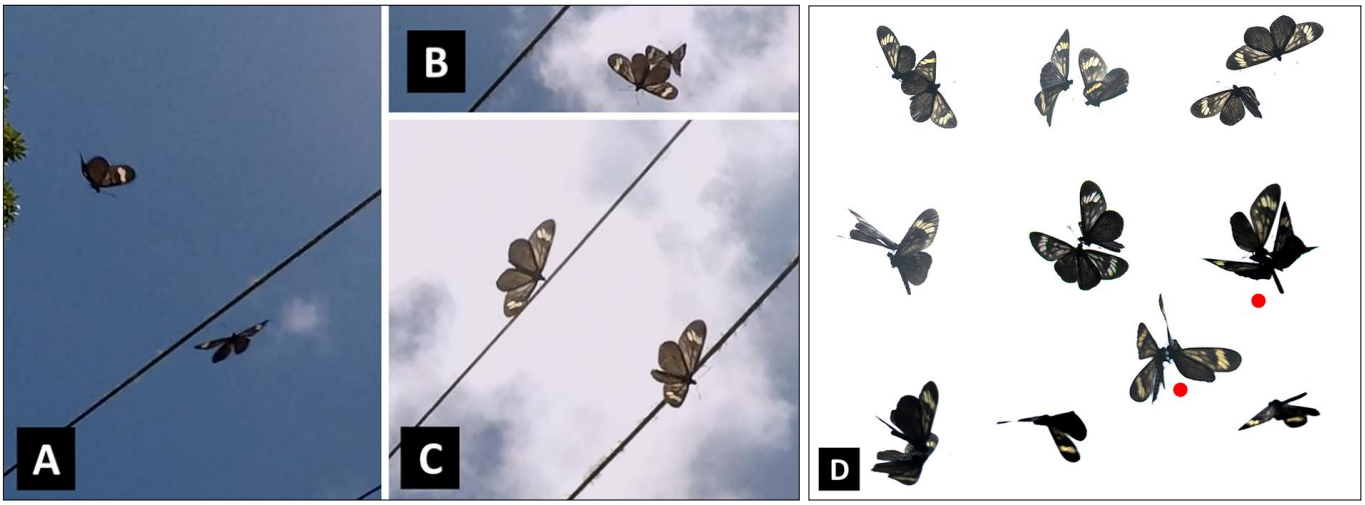


Fig 6: (A-C) A sequence of aerial chases between two A. zikani males on November 10, 2017. (D) Mosaic composed of 10 photos of the interaction between two *A. zikani* males showing details of the aerial fights. In the two photos with red dots the males use their legs to do a physical contact.

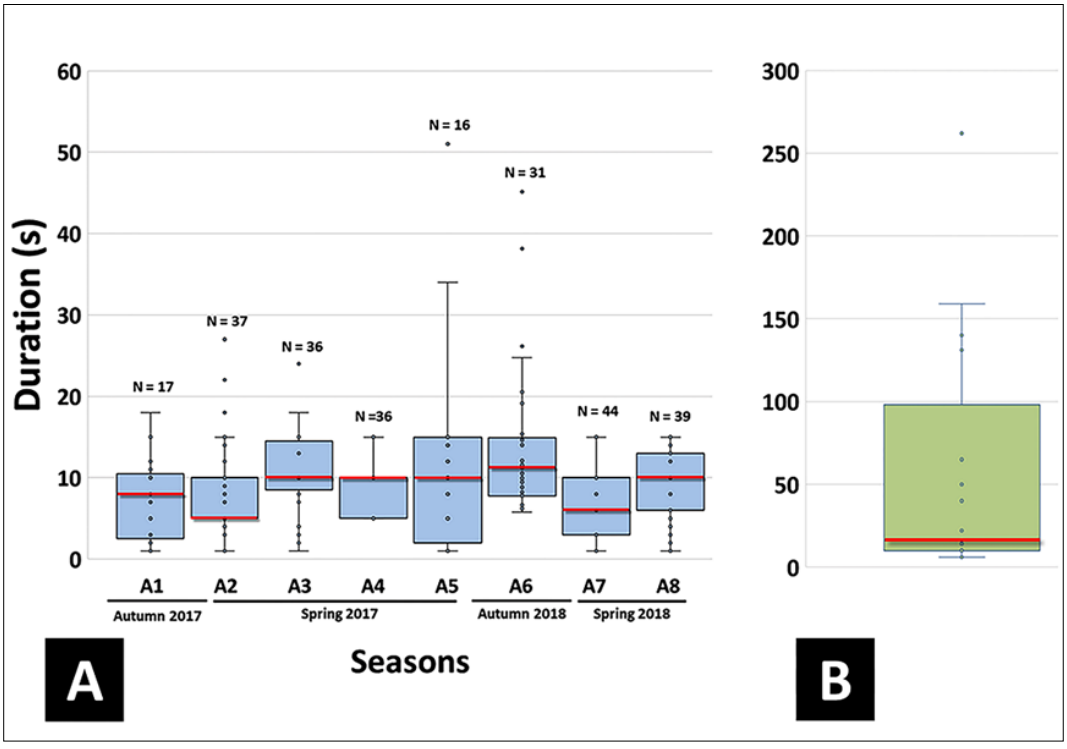
When was possible to identify the individuals the territory owner expulsed the intruder (n = 5).

Other typical chase sequence was recorded on March 28, 2017, at territory T3 which was used only in that autumn season. The owner mixed small territorial flights with landing on selected perches at 5-10 m height and, sometimes inspecting and expulsing intruders.

The chases usually determine which male owns the territory, but *A. zikani* has some particularities as the formation of adjacent linear territories side by side, without the visible presence of satellite males perched in the neighbors. When flying, the territory owner gives itself more encouragement and attacks the invader by throwing its body against its opponent. This agonistic behavior can simply expel the intruder or initiate a series of aerial encounters.

Aerial fights were recorded between males and duration of these events varied between 1 and 51 seconds (median = 10; n = 142; Fig. 7 A). Only 20 (14.08%) aerial fights were finished with both males continuing the strife in the ground substrate.

During the study, seven strifes in the ground were recorded (Table 1, FA) varying between 6 and 262 seconds (median = 16; n = 20; Fig. 7 B) but, when they fall on vegetation (n = 13) the durations were not recorded due to the difficult of to accompany the individuals inside the bush.



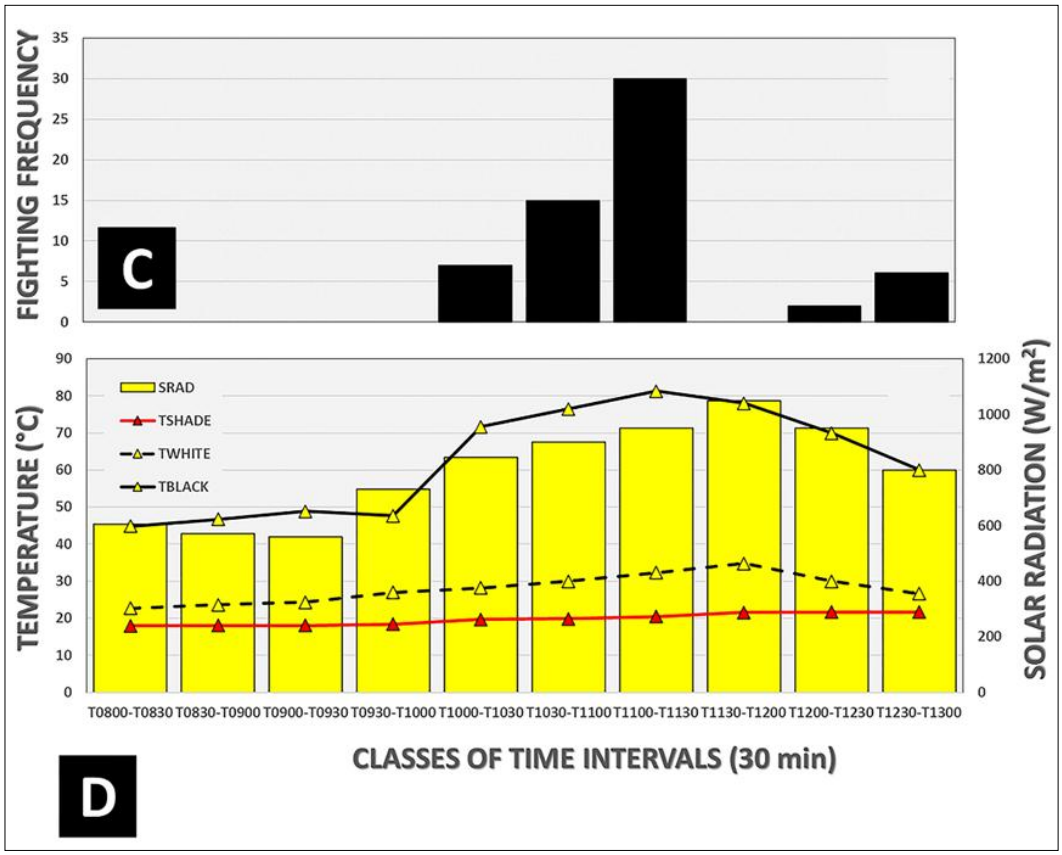


Fig 7: (A) Duration (s) of aerial encounters between males observed in eight different individuals in eight different dates. Most encounters (72.66%) had less than 10 seconds duration, the longest generally producing high vertical spiral flights. (B) Duration of strife in the substrate (ground or on vegetation). Medians in red. (C) Fighting frequency of *Actinote zikani* males during March 24, 2018, in the territories T1 and T2 in relation to (D) air temperature at shade, TSHADE in °C, solar radiation SRAD in W/m^2, and surface temperature in white, TWHITE and black, TBLACK, surfaces. In this period wind velocity was below 5 m/s.

When dropped on the ground, males used their size and mass to immobilize the opponent using also their four functional abdominal legs. Genital valves and uncus (Figs. 8 A-B) were used to infringe damages to the opponent' abdomen (Figs. 8 C-F).

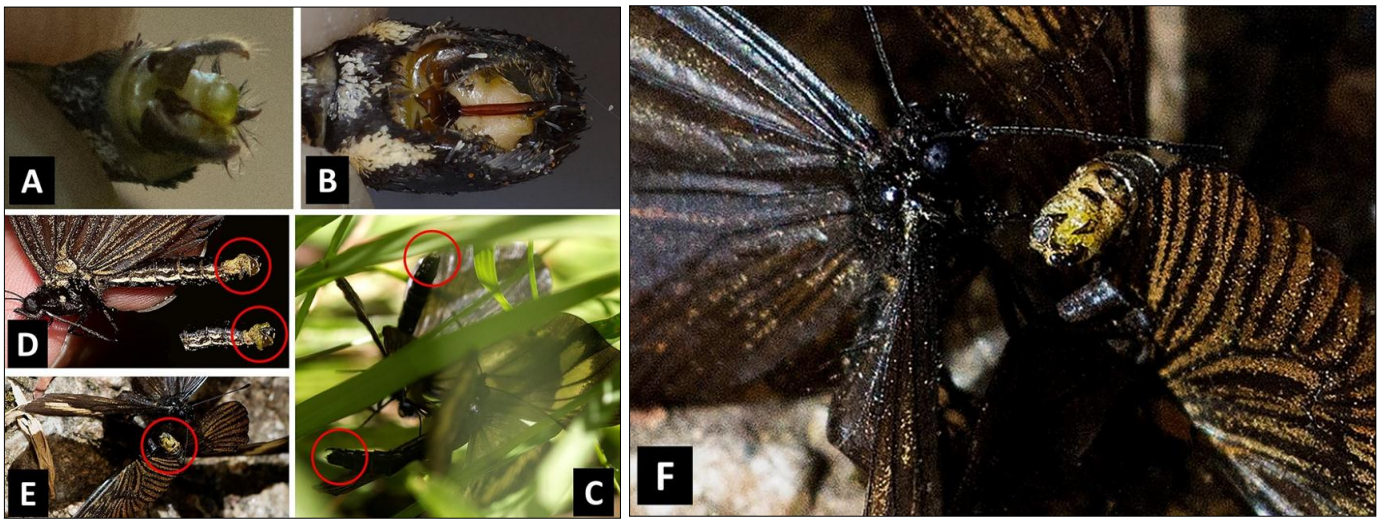


Fig 8: (A) Detail of the open valves and uncus of a new emerged male which are used to infringe damages to the opponent showing the absence of plug-forming bristles. (B) Fight in the ground between two males of *A. zikani* at main territory showing the abdominal curling used by to reach the abdominal tip of the opponent. (C): Abdomen apex of males, c1 and c2, showing that genitals were opened to pinch. (D-F) Damages in the terminal portion of male abdomen due to strife in the substrate.

In this context, genitals are used to pinch the adversary body (n = 10). The injuries caused to the opponent remove the integument covering the abdomen, exposing the fat yellow internal tissues.

After these contents, some males (n = 7) they kept tucking their abdomen during few minutes, as a response to the damages caused (Fig. 9 A).

Despite the low risks of direct injury during ground clashes the opposing males are vulnerable to the action of potential predators such as spiders (Fig. 9 B) and ants.

Flight stratification and interactions with other butterfly species

During the period when *A. zikani* is on the fly, we have observed three other *Actinote* species flying together the main territorial area: *A. parapheles*, *A. rhodope*, and *A. discrepans*. After two years of recordings, the pattern found was that there is a vertical stratification between these species (Fig. 9 A).

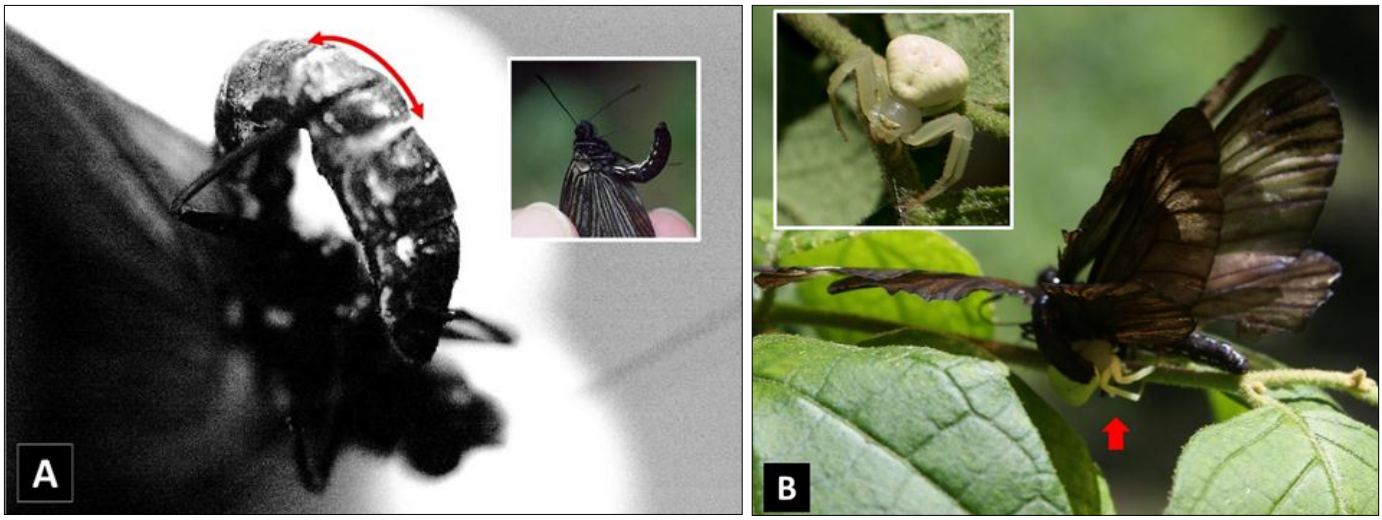


Fig 9: (A) Abdominal curling behavior of an *A. zikani* male after fighting with a conspecific April 4, 2017. (B) Two *A. zikani* males which fell on leaves to continue the fight and were predated by a crab spider (Thomisidae) at territory T4 on November 12, 2017.

These three species, together with *A. genitrix*, *A. thalia pyrrha*, and *A. carycina*, were encountered in flower patches along the dirt road to Paranapiacaba. Other butterfly species which have territorial behavior are also present occupying the upper layer h3 (Fig. 10 B-C). Others, as the large *Morpho epistrophus*, which have linear movements which reaches more than 300m (n = 5) and random flight, pass at different heights and are chased by *A. zikani* males.

Fig 10: (A) Stratification of butterfly species present in the territories T1 and T2 (main territorial area) during six flight seasons (2017-2019). (h1) *A. zikani*. (h2) *A. zikani* and *A. parapheles*. (h3) *A. discrepans* and other nymphalid butterfly species. Vertical scale in meters. In other points of the Bela Vista trail the maximum height can reach 15m but, are points where territorial males were not present (see Francini *et al*. [8] for more details). (B-C) Two other nymphalid species which also have territorial perches in h3 layer. (B) *Adelpha* sp., and (C) *Eteona tisiphone*.

Role of temperature in the territorial behavior

Fighting frequency of *A. zikani* males during March 24, 2018, at the territories T1 and T2 showed a peak when the temperatures of white and black surfaces was 34°C and 82°C, respectively (difference = 48°C) while at shade was 20°C (Fig. 7 C-D).

Wind and clouds intercepted solar radiation which reached 1,100 W/m^2 on some moments dropping quickly to 200 W/m^2 in other. From 11:30h to 12:00h AM, with sun without clouds, body heating was high and territorial activity stopped returning only after 12:00h with lesser intensity.

The males who establish their territorial flight responded quickly to the decrease in solar radiation when the sun was covered by clouds. Thus, depending on the weather conditions, the territorial flight can be continuous, without any landing, or it can be mixed with landings whenever the butterfly's body temperature is below the minimum threshold for the continuation of the flight (Fig. 11 A-C).

The temperature in the shade and the wind are two conditions that will influence this behavior. On November 18, 2018, a male was observed for 156 minutes. Perching occurred when solar radiation was low (range = 150 to 500 W/m^2; median 300 W/m^2; n = 27) and flying when it was higher (range = 200 to 1,100 W/m^2; median 300 W/m^2; n = 128). Solar radiation had a significant hole in the perching behavior (Wilcoxon rank sum test with continuity correction; W = 302.5, $p < 0.0001$; Fig. 11 D) therefore, the probability of perching decreases with the increase of solar radiation (glm binomial logit; null deviance= 143.367 on 154 degrees of freedom; $p < 0.0001$; Fig. 11 E).

The dark scales which recover *A. zikani* wings are more efficient in to store heat from solar radiation and, in some specific areas they are recovered by androconial scales. Thus, male wings have four functions: flight aerodynamics (primary), reproduction (signaling with color pattern and pheromones), thermoregulation, and protection against visual predators (also signaling with a warning coloration).

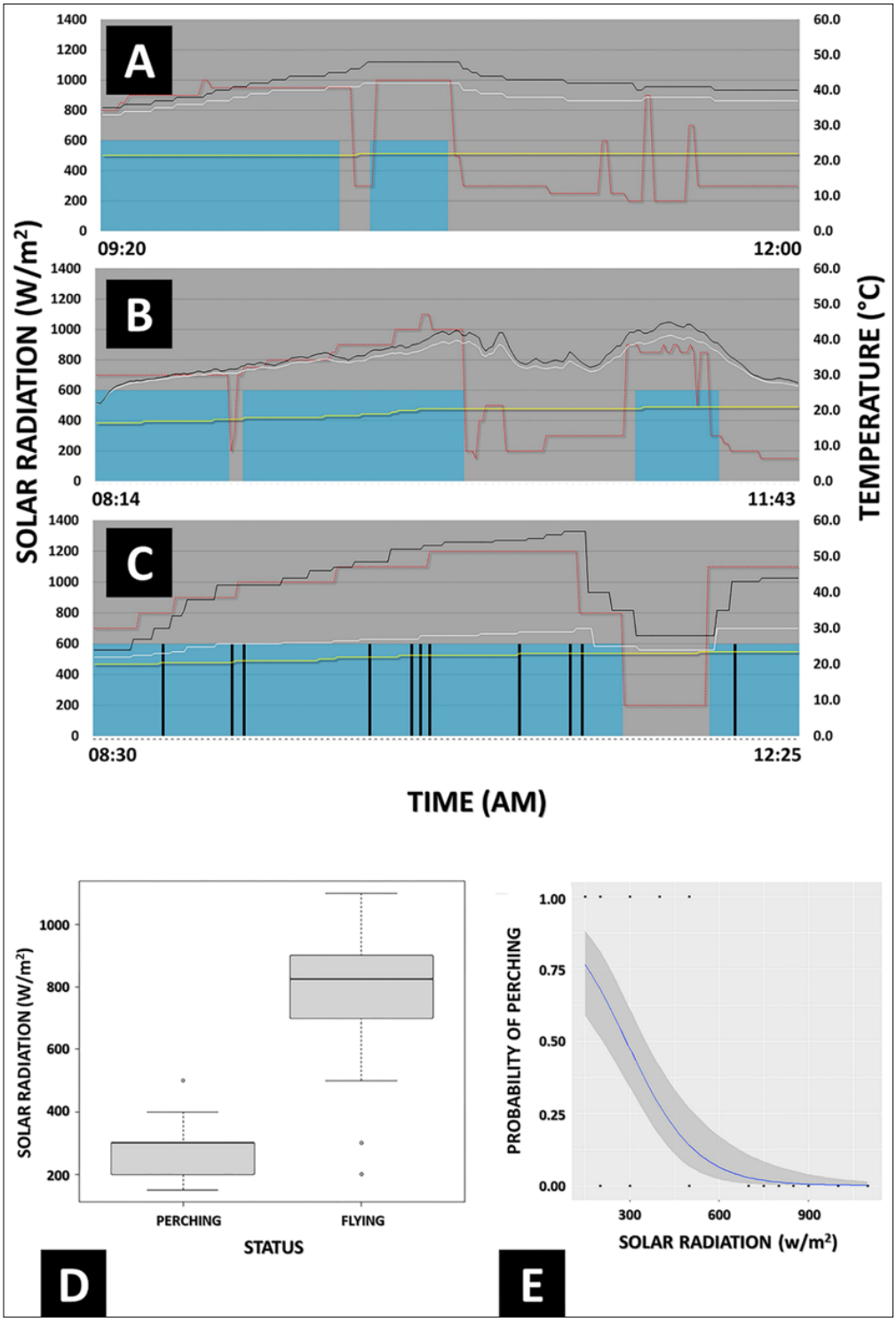


Fig 11: Time schedule of the behavior of three males recorded on different dates in territory T1. (A) April 11, 2017, at T1 from 08:20 AM to 10:50 AM. (B) October 28, 2017. (C) October 31, 2017. Yellow line indicates de temperature at shade (°C). Red line indicates the instantaneous solar radiation (W/m^2) which is lowered when sun is recovered by clouds. White and black lines indicate surface temperature of white and black polystyrene sponge surfaces. Blue area indicates when the territory owner is flying. Black bars are events of fighting against intruders. (D) Status of a male that was observed for 156 minutes on November 18, 2018, showing its behavioral responses to the variation of solar radiation. (E) Decrease of the probability of perching with the increase of solar radiation.

Multiple marking and release (MMR)

In six dates during spring generation of 2017, an MMR effort was proceeded, marking 61 individuals, most between TOWER 2 and TOWER 3 (Fig. 12 A-B).

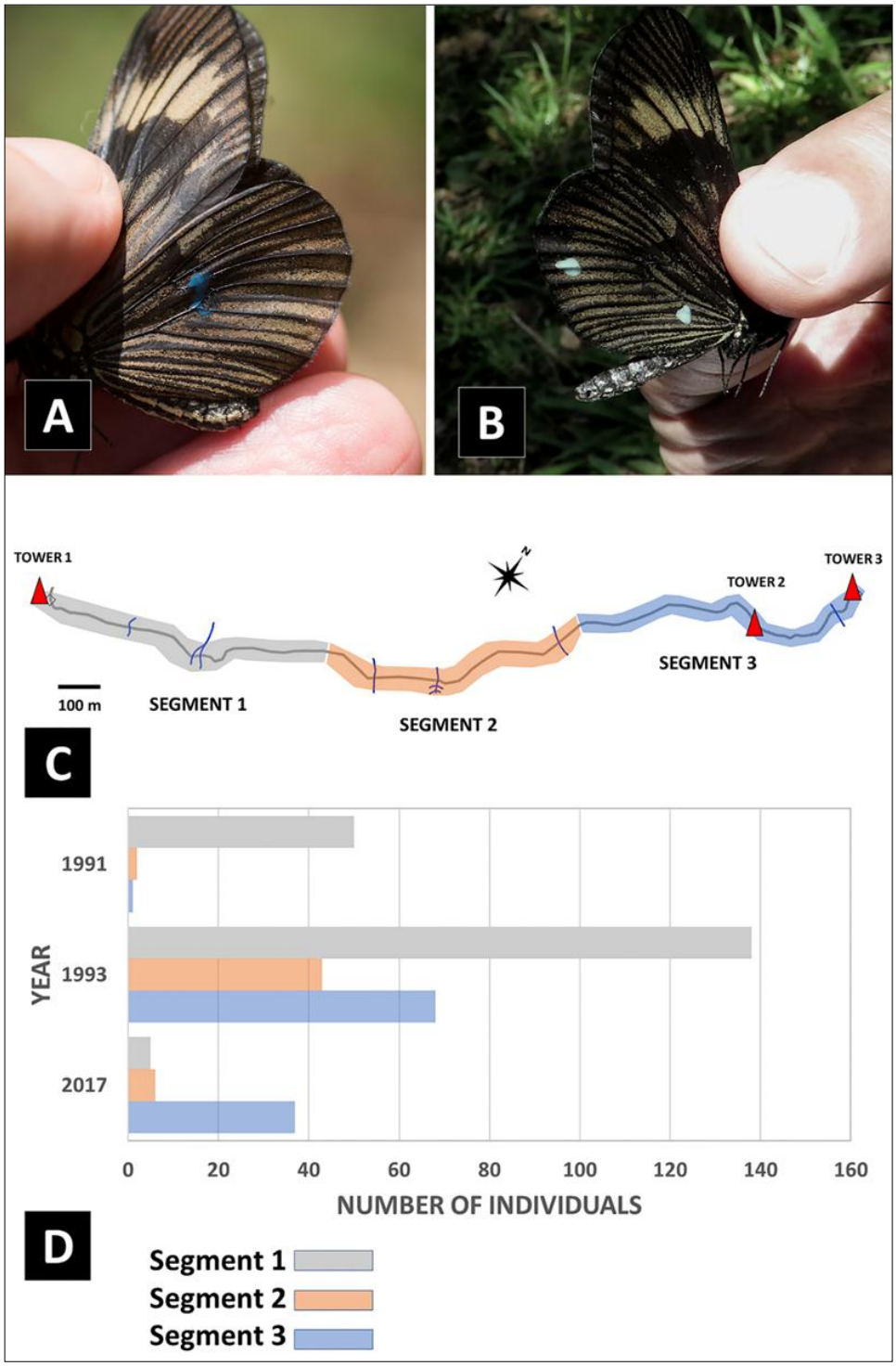


Fig 12: Two *A. zikani* adults after marking. (A) Female F03, marked on October 10, 2017, and male 28, marked on November 3, 2017. (B) Scheme of the Bela Vista trail showing the division in three equal segments of approximately same length to compare (C) the number of sighted individuals in three different years.

Only three males were recaptured, one, one time, one two times and male #6, five times. This male, marked at territory T4 on October 28, fighting simultaneously with males #6 and #8, was recorded (recaptured) when in ground strife with another male on November 3, at territory T1, being identified by the blue spot in the left hindwing. A residency time of at least seven hard days. The MMR sex-ratio was strong male biased (0.92:0.08) also in the records of sighted individuals in six generations (Table 1 TOTAL, SR).

Discussion

Male hilltopping and territoriality

The hilltoping and territorial behavior (2016-2019) was not different of the records of 1991, 1993, and 1994 [1]. Four males were recorded flying throughout the territory T1 during more than two hours without landing, inspecting agonistically other intruder insects.

Differences in population densities may be important in the butterfly hilltopping and territorial behavior [18-19]. This reinforces the idea of territory as a meeting place, which may include hilltopping which was observed by other authors [18, 20]. However, *A. zikani* female behavior and mating will be studied in another paper.

Males of other butterflies as *Heraclides thoas brasiliensis*,

Battus polydamas polydamas, and *Eurytides orthosilaus* (Papilionidae) share the same behavior establishing territories in fixed relative positions (e.g., toward the sun or magnetic north) and occupying the same areas during several weeks where mating sites are formed [21]. These characteristics including patrolling of delimited areas combined with intolerant behavior in relation of other males, which can lead to chases and physical clashes on the ground, appear to characterize territory interactions which usually determine who male owns the territory [21-22].

Males are probably fighting only for mating areas and not for territories that contain resources used by females [22-23-24]. Strong defense of hilltopping territories were recorded for *Vanessa atalanta* in a site in Arizona and that tenacity of defense was dependent of the density of conspecific males [24] the only difference with our results was that territories were formed after 02:00 PM. The mating system of butterflies which fit to this behavior was denominated as lek polygyny because the unique resource in their territories is the eventual presence of females [19].

Parker [25] created the concept of resource holding potential (RHP) which is the capacity of individuals to hold its resource as selective advantage. Therefore, individuals with higher RHP have advantage over its opponents [26] and this is correlated with owners' capacity to inflict damages to the opponent body size [27]. Both, size and mass, are characteristics that have been used to explain the why an individual male butterfly has a high RHP.

It is possible that this unique *A. zikani* population is harmed by low densities of food resources and individuals, besides the deaths that occur precisely in the territories due to confrontations and mating, reveal excessive costs for the future stability and viability of this population. However, this strategy involving a mating point at the highest points of the landscape can mitigate the effects of impacts due to low densities of individuals and food resources and have been observed in other butterfly populations.

Despite these advantages, hilltops are more prone to stronger winds and not always offer suitable nectar resources size [28].

Persistency and resiliency in territory and the use of perches for resting or basking

This behavior appears to indicate that the maintaining early in the territorial area increases the probability of to obtain a mate (see Takeuchi & Honda [29]). The perches serve as surveillance platforms from which they can observe intruders, like *A. pellenea* (RBF unpublished data and [22]). It is noted that "restricted patrolling" and "perching" in the butterflies *Actinote pellenea* [22] and *Archonias* [23, 30], can be considered true territorial behaviors.

Flight behavior, aerial chases, and strife in the substrate

In butterflies, flight has important functions in their survival [31-32] and it is not different for *A. zikani*. Like Papilionidae species [20] and *Actinote pellenea* [22], clashes between *Actinote zikani* males on the ground seem quite aggressive using tarsal claws and valves (genitalia) against the opponent's wings and body.

Considering that *Actinote* are aposematic butterflies with flexible wings and exoskeleton, any crashes on the ground apparently does no cause direct physical damages. However, the butterfly *Archonias brassolis* that also present territorial behavior does not take their combats to the ground [30], maybe due it is very fragile, which would lead to the loss of legs or breakage of wings, thus overcoming any cost for the defense of the territory.

Until now, is assumed that butterflies do not have weapons or organs such as teeth, nails, or horns with which they use to injure their opponents [26]. However, the clashes between males of A. zikani during the ground phase points to distinctive behavior, when the males try to hit the opponent using their valves and uncus.

In the territorial clashes of *Actinote pellenea* males [22], and RBF, unpublished data (n = 12), *A. brylla* (RBF, unpublished data, n = 4), and *A. parapheles* (RBF, unpublished data, n = 2), there were no records of physical injuries in the contenders.

Flight stratification and interactions with other butterfly species

Previous works showed that, in some Nymphalidae assemblages the species are vertically stratified by height of flight [31-37].

Role of temperature in the territorial behavior

Insect wings are not constituted only by a lifeless cuticle and have biophysical properties that ensure the wing functionality for the flight which is their main function [38, 40]. Their wings have several bristles which are mechanoreceptors that are used in flight control and are recovered by androconial scales which are used for the identification of conspecifics [39].

Multiple marking and release (MMR)

In 2017 MMR, this low number of male recaptures (0.05%) was significant different from 1993 MMR: 5.3% in spring, and 5.7% in autumn meaning that the residency in the population was short [1]. Maximum residency time of *Actinote* species was low: six days for *A. zikani* of 1993 spring generation, 10 days for *A. mamita mitama*, and 13 days for *A. pellenea pellenea*. However, in 16 Neotropical butterfly species studied, few can reach to 162 days (see Table 3 in Francini [41]).

In the past, recorded sex-ratios along the Bela Vista trail were: 0.82:0.18, in autumn 1991, 0.75:0.25, in spring 1993, and 0.96: 0.04, in autumn 1994, all male biased [1]. The distribution of individuals in this area was also different in these dates (Fig. 12 C-D).

As in other *Actinote* species which have strong protandry [42], *A. zikani* males emerge before females [1]. This is a male reproductive strategy which results from competition for mates and should primarily occur in species maintaining female monogamy [43].

Final considerations

In our study, the observations of seven generations of *A. zikani* (2016 to 2021) showed the continuous use, generation after generation, of the same main territory (T1-T4).

From the environmental parameters collected, the amount of solar radiation and protection against stronger winds were the main characteristics of this area. In these territorial areas, even in seasons where few floral resources were available, no individuals of this species were observed foraging on flowers or inflorescences.

Regarding the energy issue, *A. zikani* may be suffering due the lack of food resources for adults, observations about the low availability of flowers and due to records of sudden deaths during clashes generate speculation about fragility or lack of energy considering that the patrolling and disputes for

territory and females is untiring.
In the present study, using their genitalia, males of *A. zikani*, broke the paradigm that butterflies have no mechanical weapons to infringe damages in other individuals.

Conclusions
All this knowledge allows us to create some questions that need to be answered in future research: (a) Could it be that intraspecific recognition mechanisms are weak and do males need to make physical contact with the potential partner to mate? This would explain the reason for the partial similarity between the clashes between males and mating behavior with females. (b) When a female is harassed two responses to male are possible: to accept or to reject it. However, we need to consider that for to reject the male, the female needs to gain freedom only using her legs, abdomen, and wings. (c) When two males are in strife in the substrate both have the same weaponry and the same innate behavior to manage and subdue the female, therefore the results may be bad for one or both. (d) Could the absence of male genital plug-forming bristles in some *Actinote* species which sphragis were not external increases the force used to hold females (and consequently) males? This will increase the time and the power of damages in the opponent's body.

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Conflict of interest
The authors declare that they do not have any conflict of interest.

Author's contribution
RBF designed the project. All authors collected data in the field. RBF analyzed the data and wrote primary draft of the manuscript. RRR, IG, and EFSF provided additional suggestions for the final manuscript.

References
1. Francini RB, Freitas AVL, Jr KSB. Rediscovery of *Actinote zikani* (D'Almeida) (Nymphalidae, Heliconiinae, Acraeini): Natural history, population biology and conservation of an endangered butterfly in SE Brazil. Journal of the Lepidopterists' Society. 2005;59(3):9.
2. Francini RB, Freitas AVL, Penz C. Two new species of *Actinote* (Lepidoptera, Nymphalidae) from Southeastern Brazil. Zootaxa. 2004;719(1):1.
3. Freitas AVL, Francini RB, Mielke OHH, Rosa AHB, Magaldi LM, Silva-Brandão KL. A new and rare *Actinote* Hübner (Lepidoptera: Nymphalidae: Heliconiinae: Acraeini) from Southeastern Brazil. Neotropical Entomology. 2020 Oct;49(5):696-703.
4. Francini RB, Penz CM. An illustrated key to male *Actinote* from Southeastern Brazil (Lepidoptera, Nymphalidae). Biota Neotrop [Internet]. 2006 [cited 2019 Dec 27];6(1). Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1676-06032006000100013&lng=en&tlng=en
5. Brown KS, Francini RB. Evolutionary strategies of chemical defense in aposematic butterflies: cyanogenesis in Asteraceae-feeding American Acraeinae. Chemoecology. 1990;1(2):52–6.
6. Francini RB, Ramos RR, Grantsau I, Freitas AVL. Resultados parciais do projeto de pesquisa sobre a borboleta *Actinote zikani* e sua planta de alimento larval, *Mikania obsoleta*, no Parque Natural Municipal Nascentes de Paranapiacaba, Santo André, SP. Santos, SP: Prefeitura Municipal de Santo André, 2017, 91.
7. Shields O. Hilltopping. Journal of Research on the Lepidoptera. 1967;6(2):69–178.
8. Scott JA. Hilltoping as mating mechanism to aid the survival of low density species. Journal of Research on the Lepidoptera. 1968;7(4):191–204.
9. Wilson EO. Sociobiology. Abridged ed. Cambridge, Mass: Belknap Press of Harvard University Press; 1980, 366.
10. Francini R, Ramos R, Grantsau I. Higher and hotter: the continuous use of a territorial area by males of the threatened butterfly, *Actinote zikani* (Nymphalidae: Heliconiinae: Acraeini) in a Serra do Mar hilltop. Journal of Entomology and Zoology Studies. 2022;10(4):31–8.
11. Lillywhite HB. Behavioral Temperature Regulation in the Bullfrog, *Rana catesbeiana*. Copeia. 1970;1970(1):158.
12. Lillywhite HB, Friedman G, Ford N. Color Matching and Perch Selection by Lizards in Recently Burned Chaparral. Copeia. 1977;1977(1):115.
13. Van Dyck H, Matthysen E. Thermoregulatory differences between phenotypes in the speckled wood butterfly: hot perchers and cold patrollers? Oecologia. 1998;114(3):326–34.
14. R Development Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2022.
15. RStudio.com. RStudio desktop. RStudio.com; 2022. Available from: https://www.rstudio.com/products/rstudio/
16. Wood. Package 'ggplot2'. Create Elegant Data Visualisations Using the Grammar of Graphics. V. 3.3.6 [Internet]. 2022. Available from: https://cran.r-project.org/web/packages/ggplot2/ggplot2.pdf
17. Wood S. Package "MGCV": Mixed GAM Computation Vehicle with Automatic Smoothness Estimation. V. 1.8-40 [Internet]. 2022. Available from: https://cran.r-project.org/web/packages/mgcv/index.html
18. Alcock J. Hilltopping in the Nymphalid Butterfly *Chlosyne californica* (Lepidoptera). American Midland Naturalist. 1985;113(1):69.
19. Alcock J, Dodson G. The diversity of mating systems of hilltopping insects. American Entomologist. 2008;54(2):80–7.
20. Alcock J. Leks and hilltopping in insects. Journal of Natural History. 1987;21(2):319–28.
21. Pinheiro CEG. Territorial hilltopping behavior of three swallowtail butterflies (Lepidoptera, Papilionidae) in Western Brazil. Journal of Research on the Lepidoptera. 1991;29(1–2):134–42.
22. Carvalho MRM, Peixoto PEC, Benson WW. Territorial clashes in the Neotropical butterfly *Actinote pellenea* (Acraeinae): do disputes differ when contests get physical? Behav Ecol Sociobiol. 2016;70(1):199–207.
23. Francini RB, Sobral-Souza T, Filho S, Ramos RR. Territorial behavior of the butterfly *Archonias brassolis tereas* (Godart, 1819) (Lepidoptera: Pieridae: Pierinae) in three sites in Southeastern Brazil. International Journal of Fauna and Biological Studies. 2019;6(6):34-40.

24. Brown WD, Alcock J. Hilltopping by the red admiral butterfly: mate searching alongside congeners. The Journal of Research on the Lepidoptera. 1991;29(1–2):1–10.
25. Parker GA. Assessment Strategy and the Evolution of Fighting Behaviour. Journal of Theoretical Biology. 1974;47(1):223-43.
26. Takeuchi T. Agonistic display or courtship behavior? A review of contests over mating opportunity in butterflies. J Ethol. 2017;35(1):3-12.
27. Arnott G, Elwood RW. Assessment of fighting ability in animal contests. Animal Behaviour. 2009;77(5):991-1004.
28. Painter KJ. Multiscale models for movement in oriented environments and their application to hilltopping in butterflies. Theor Ecol. 2014;7(1):53-75.
29. Takeuchi T, Honda K. Early Comers Become Owners: Effect of Residency Experience on Territorial Contest Dynamics in a Lycaenid Butterfly. Ethology. 2009;115(8):767-73.
30. Francini R, Sobral-Souza T. Trade-off between mass allocation and perching behavior in the pierid butterfly *Archonias brassolis tereas* (Godart, 1819) (Lepidoptera: Pieridae: Pierinae). Journal of Entomology and Zoology Studies. 2017;5(1):602-8.
31. DeVries PJ, Penz CM, Hill RI. Vertical distribution, flight behaviour and evolution of wing morphology in *Morpho* butterflies: Wing evolution in *Morpho* butterflies. Journal of Animal Ecology. 2010;79(5):1077–85.
32. Graça MB, Pequeno PACL, Franklin E, Morais JW. Coevolution between flight morphology, vertical stratification and sexual dimorphism: what can we learn from tropical butterflies? J Evol Biol. 2017;30(10):1862-71.
33. Beccaloni GW. Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host-plant height. Biological Journal of The Linnean Society. 1997;62(3):313–41.
34. Hill J, Hamer K, Tangah J, Dawood M. Ecology of tropical butterflies in rainforest gaps. Oecologia. 2001;128(2):294–302.
35. Fermon H, Waltert M, Mühlenberg M. Movement and vertical stratification of fruit-feeding butterflies in a managed West African rainforest. Journal of Insect Conservation. 2003;7(1):7–19.
36. Fermon H, Waltert M, Vane-Wright RI, Hlenberg MM. Forest use and vertical stratification in fruit-feeding butterflies of Sulawesi, Indonesia: Impacts for conservation. Biodiversity and Conservation. 2005;14(2):333–50.
37. Mena S, Kozak KM, Cárdenas RE, Checa MF. Forest stratification shapes allometry and flight morphology of tropical butterflies. Proc R Soc B. 2020;287(1937):20201071.
38. Pass G. Beyond aerodynamics: The critical roles of the circulatory and tracheal systems in maintaining insect wing functionality. Arthropod Structure & Development. 2018;47(4):391–407.
39. Barth R. Os orgaos odoriferos dos lepidopteros. Boletim do Parque Nacional do Itatiaia. 1960;7:1–159.
40. Bladon AJ, Lewis M, Bladon EK, Buckton SJ, Corbett S, Ewing SR, *et al*. How butterflies keep their cool: Physical and ecological traits influence thermoregulatory ability and population trends. Zipkin E, editor. Journal of Animal Ecology. 2020;89(11):2440–50.
41. Francini RB. Role of age and weather on residence time of males of *Anartia amathea* roeselia butterfly in a subtropical area in Southeastern Brazil. International Journal of Fauna and Biological Studies. 2018;5(3):217–27.
42. Francini RB. Biologia e ecologia das borboletas *Actinote* (Lepidoptera: Nymphalidae, Acraeinae) do sudeste do Brasil [Master dissertation]. [Campinas]: Universidade Estadual de Campinas - UNICAMP; 1989.
43. Wiklund C, Fagerström T. Why do males emerge before females?: A hypothesis to explain the incidence of protandry in butterflies. Oecologia. 1977;31(2):153–8.