MATING BEHAVIOR IN TWO SYMPATRIC SPECIES OF ANDEAN TIGER BEETLES (CICINDELIDAE)

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RESUMEN

Comportamiento de apareamiento de dos especies simpátricas de escarabajos tigres.- El género *Pseudoxycheila* de escarabajos tigre (Cicindelidae) está conformado por 21 especies distribuidas a elevaciones medias y altas de los Andes desde Bolivia hasta Venezuela y en las montañas en Panamá y Costa Rica. El centro de origen del género está en los Andes de Colombia y Ecuador. En este estudio describimos el comportamiento de apareamiento de dos especies simpátricas, *P. chaudoiri* y *P. confusa*, encontradas a una elevación de 1900 m en la cordillera Occidental de Colombia (3° 30' N 76° 34' O). Las dos especies se encuentran en hábitats similares, sobre superficies inclinadas de suelo arcilloso desnudo, pero se segregan espacialmente por diferencias microclimáticas. El comportamiento reproductivo de ambas especies fue similar. Machos y hembras se encontraron de manera aleatoria en el área de postura de huevos y el macho intentó montar a la hembra. En los casos en los que la hembra no estaba poniendo huevos, esta intentó resistirse en un forcejeo pre-cópula, a lo cual siguió la cópula, una asociación post-copulatoria (APC), un forcejeo post-cópula y la separación final de la pareja. Aproximadamente la mitad de los intentos de apareamiento ocurrieron con hembras que estaban empezando a poner; en estos casos no hubo forcejeo pre-cópula y generalmente al terminar la cópula la hembra prosiguió poniendo el huevo con el macho en amplexus. La APC probablemente representa un comportamiento de guarda de la pareja, pero algunos machos en APC fueron desmontados por otros machos. Sin embargo, en algunos casos los machos en APC probablemente pudieron fertilizar varios huevos que fueron puestos en secuencia. El comportamiento de apareamiento de estas dos especies posiblemente refleja un conflicto sexual de intereses, pues los machos están generalmente buscando aparearse y las hembras se resisten, pero solo hasta el punto en que resistirse o interrumpir el proceso de postura les resulta más costoso que aceptar al macho.

Palabras clave: Cicindelidae, comportamiento de apareamiento, especies simpátricas, escarabajos tigre, Pseudoxycheila, segregación de microháhitat.

SUMMARY

The tiger beetle genus *Pseudoxycheila* (Cicindelidae) currently contains 21 species, distributed in mid and high elevations in the Andes from Bolivia to Venezuela and in mountains in Panama and Costa Rica. The center of origin of the genus is in the Andes of Colombia and Ecuador. In this study we describe the mating behavior of two species, *P. chaudoiri* and *P. confusa*, that co-occur at an elevation of 1900 m on the western Andean range of Colombia (3° 30' N 76° 34' W). The two species used similar habitats, which are inclined surfaces with bare, clay soils, but were spatially segregated by microclimatic differences. The mating behavior of both species was similar. It was characterized by haphazard encounters of males and females at oviposition sites, and males attempting to mount females. When females were not laying, mounting was followed by a pre-copulatory struggle (female attempting to dislodge male), copulation, a post-copulatory association (PCA), a post-copulatory struggle, and finally dislodging of the male by the female. About half of the mating attempts occurred with females that were starting to lay eggs; in theses cases usually there was no

pre-copulatory struggle and after copulation the female usually continued egg laying with the male in amplexus. PCA likely represented mate-guarding behavior, but males in PCA were dislodged by intruding males. In some cases, however, males in PCA were probably able to fertilize several eggs that were laid in sequence. The mating behavior of these two species is possibly a result of a sexual conflict of interests, in which males try to mate with any female they encounter and females resist, but only to the point at which struggling and interrupting egg-laying is more costly than accepting copulation.

Key words: Cicindelidae, mating behavior, microhabitat segregation, Pseudoxycheila, sympatric species, tiger beetles.

INTRODUCTION

Female tiger beetles (Cicindelidae) oviposit one egg at a time in small burrows that they dig in bare soil (Pearson & Vogler 2001). Presumably, females carefully select the exact place to dig a burrow, as the larva will spend up to one year and pupate there (Palmer 1976). Depending on local conditions, tiger beetles are active for only a few hours around noon, when the thermal environment is appropriate. Females of *Pseudoxycheila tarsalis* spend a large portion of this time probing the soil with the tip of the abdomen, apparently searching for adequate places to oviposit, and lay only one or two eggs in a single day (Palmer 1976).

Mate-guarding behavior, in which males remain mounted on the back of females after insemination, has been observed in several species of tiger beetle (Pearson & Vogler 2001). Because mating occurs in the oviposition area, and females remain there for several hours, there is a high potential for competition among males (Alcock 1994). Mate guarding may prevent other males from mating with the female, as these males may dislodge the spermatophore inserted in a previous mating. Males have been observed to repeatedly insert the aedagus, presumably in an attempt to dislodge previous sperm (Pearson & Vogler 2001, Rodriguez 1998). In addition, although the mechanism of sperm precedence is unknown for tiger beetles, a guarding male would presumably increase his chances of fertilizing the eggs if he was the last to mate with the female.

The Neotropical genus *Pseudoxycheila* contains 21 species distributed at mid and high elevations in the Andes of Bolivia, Peru, Ecuador, Colombia and Venezuela and in mountains in Panama and Costa Rica. *Pseudoxycheila* spp. are medium to large-sized tiger beetles, green or blue-colored with a yellow or orange dot on each elytron, and small eyes. A recent taxonomic revision of the genus (Cassola 1997) proposes that Colombia and Ecuador, with 10 and 11 species respectively, are the center of origin. Three species (*P. macrocephala*, *P. colombiana* and *P. confusa*) are endemic of Colombia.

At middle elevations (1800-2000 m) in the western range of the Colombian Andes, two species of *Pseudoxycheila*, *P. confusa* Cassola, 1997 and *P. chaudoiri* Dokhtouroff, 1882, occur in sympatry. These tiger beetles inhabit open and sunny areas with clay soils, on inclined banks along streams and trails. The objectives of this paper are 1) to describe the mating behavior of *P. confusa* and *P. chaudoiri*, and 2) to determine whether there are any segregation mechanisms that prevent hybridization between these two closely related species.

STUDY AREA AND METHODS

The study was conducted at Finca Zíngara, 4 km north of El Dieciocho (Km 18 of the Cali-Buenaventura road), at an elevation of 1900 m on the western Andean range of Colombia (3° 30' N 76° 34' W). We dissected the genitalia of 15 males collected throughout the study area to establish species identity. Species were identified according to Cassola's (1997) monograph on *Pseudoxycheila*. The two species were easily separated in the lab by their genitalia and in the field by color, size of yellow spots, and shape of elytra.

Observations on *P. chaudoiri* were made in a 30 m long x 1.8 m high bank of bare soil in a pasture. We made 18 observation sessions between 10:00 and 14:00, throughout September and October 2001. We marked the beetles by sticking a number on the elytra; because every day there were new, unmarked beetles, one hour before observations started we marked the new individuals. We also

measured with calipers (precision 0.01 mm) the length of the left elytron of each beetle, as an index of body size. In this bank we selected a 3 m stretch to focus our observations. The reproductive behaviors (mating, oviposition) of every individual observed in the focal area (usually no more than six individuals) were recorded in detail; when a beetle left the focal area no more observations were made on that individual. We also made periodic observations throughout the entire bank to determine individual movements.

Observations on *P. confusa* were made in a small (2 m x 2 m) bank inside a forest patch. Observations were made during eight sessions between April and June 2001 on marked and measured individuals (same methodology as described above). The mating and oviposition behaviors of every individual observed in this area were also recorded between 10:00 and 14:00 hours. In addition, we made six observation sessions in a 230 m trail inside the forest, to obtain data on movements of marked beetles.

At both focal study areas, we took air temperature in the shade every 15 min during each observation session, to obtain mean daily temperatures during the time beetles were active.

RESULTS

Both beetle species were found in similar habitats, on inclined surfaces with bare red (lateritic) soils on roadsides and along forest trails. Although aggregations of both species were found in close proximity, interspecies matings or other interactions were never observed. The two species seemed to be separated by microhabitat differences. Individuals of *P. chaudoiri* were observed along roadsides and trails in open, sunny areas in pastures. The mean daily air temperature at these sites was 20.7 °C (± SD = 0.9; range 19.6-22.4 °C). In contrast, *P. confusa* aggregations were observed along shaded trails with an overhead canopy. Mean daily air temperature at these sites was cooler (17.6 ± 1.1°C; range 16.1-18.5 °C). These temperatures were significantly different (Mann-Whitney U-test, P = 0.006).

Sex ratio and movement
Male and female *P. chaudoiri* and *P. confusa* were observed at oviposition sites resting, totally or partially exposed to the sun, or patrolling the area in quick and intermittent bouts of activity.

P. chaudoiri.- During 18 daily visits to the study area (spread over 32 days), a total of 49 males and 39 females were marked. This sex ratio was not significantly different from 1:1 (χ^2 = 1.14, df =1, P = 0.28). Mean size of the left elytra was 9.91 (±1.02) mm (range 9.2-10.8 mm, N = 49) for males and 9.85 (±0.34) mm (range 9.2-10.8 mm, N = 39) for females. A mean of 9.2 (±3.3) males and 6.8 (±2.9) females were observed per visit. Twenty-nine percent of males and 28 percent of females were observed in only one observation session. However, a male and a female stayed for as long as 18 and 11 days, respectively. Most individuals did not move over distances larger than 1 m wi-thin the oviposition area in their daily activities. Maximum distances moved in one day were 8 m for a male and 4 m for a female.

P. confusa.- During eight observation sessions, 13 males and 22 females were marked. This sex ratio was not significantly different from 1:1 (χ^2 = 1.16, df =1, P = 0.28). Mean size of the left elytra was 10.34 (±0.24) mm (range 9.9-10.7 mm, N =13) for males and 10.21 (±0.41) mm (range 9.7-11 mm, N = 22) for females. The mean numbers of individuals per observation session were 2.9 (±1.0) males and 3.9 (±2.0) females. Although most individuals (69% of males and 68% of females) were observed in only one observation session, the maximum number of days that a male and a female were observed in the same place were 78 and 31 days, respectively.

During six days of sampling along a 230 m trail, 34 males and 29 females of *P. confusa* were marked. Males moved an average distance of 1.6 (±1.2) m per day, whereas females moved 3.4 (±3.3) m per day. One male was observed in the same location after 64 days. In contrast, seven days was the maximum time that a female was observed in the same place.

Mating and egg-laying behavior
For both *P. chaudoiri* and *P. confusa*, males and females appeared to encounter each other by chance. Every time a male was closer than 15 cm to a female, he approached her and tried to mount her by grabbing her thorax with his mandibles. The female usually responded by running, rolling on her back, or pushing the male with her legs in an attempt to dislodge him from her back.

P. chaudoiri.- Mating behavior of this species had four phases: pre-mating struggle, copulation, post-copulatory association (PCA) and post-mating struggle. We observed 48 mating attempts, approximately half of which occurred with females that were resting or walking on the oviposition site (Figure 1). Seventy-eight percent of such attempts were successful, even though females always resisted at first. Only 25 percent of such copulations were followed by a PCA and we never observed females laying eggs during these associations (Figure 1). Two of the 48 pairs were attacked by another male. The aggressor mounted the pair and struggled for a few seconds. In both cases the riding male was displaced by the attacking male, which took its place and copulated with the female.
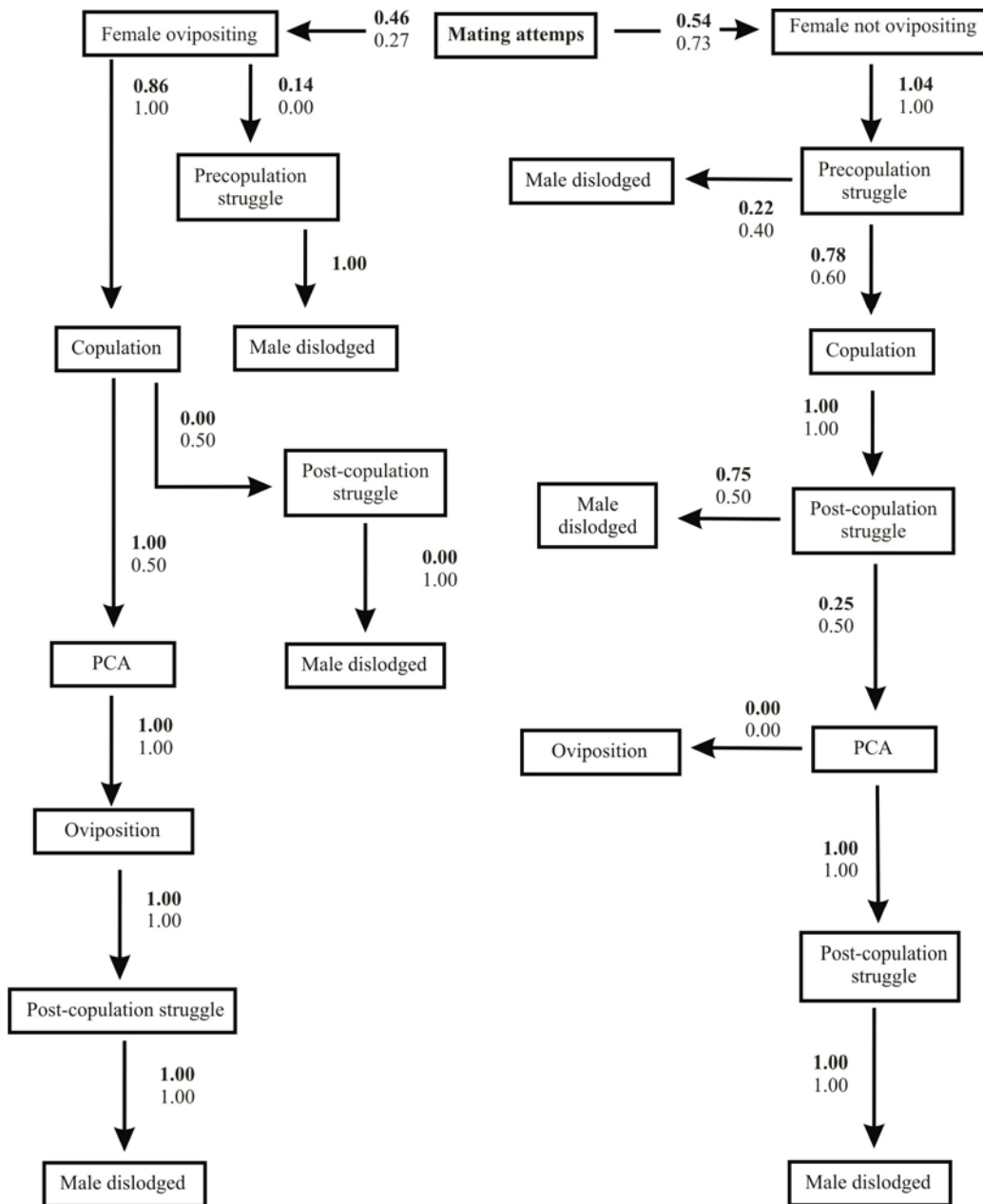

Figure 1. Flow diagram showing stages in the mating and egg-laying behavior of two species of *Pseudoxycheila* tiger beetle in the Colombian Andes. Numbers indicate the proportion of observations for each transition. Observations for *P. chaudoiri* (N = 48) in bold and for *P. confusa* (N = 15) in plain text.

Forty-six percent of the mating attempts occurred with females that were digging holes for oviposition (Figure 1). Typically, the female did not struggle and the male inserted the aedagus immediately after mounting her. After copulation, the female resumed egg-laying with the male in amplexus. Although in most cases the female forcefully caused the male to dismount after egg-laying had taken place, two females laid 2 and 3 consecutive eggs, respectively, with the male in PCA (Figure 1).

Copulation consisted of a single insertion of the aedagus that lasted 3.2 (±0.7) min (range 2.8-4.0 min, N = 12) and was followed by a PCA of variable duration, with a mean of 7.7 (±7.3) min (range 1-20 min, N = 10). The PCAs were always ended after a struggle initiated by the female (Figure 1). The two males that remained in PCA for more than 20 min, copulated a second time. Again, mating ended after a struggle initiated by the female Duration of the mountings was not related to male size (length of left elytron; Pearson correlation: R= -0.129, P= 0.69, N = 13).

Females laid eggs on inclined surfaces of bare soil along trails. Females usually dug in different spots before laying. Egg-laying was considered complete when the female covered the hole with dirt. Of 119 observations of females digging holes, in 60 percent the process was completed. Females laid as many as four eggs in one day (Table 1). The whole oviposition process (digging, actual egg-laying and covering the hole) lasted 11.9 (±3.7) min (range 6-19 min, N = 18).

P. confusa.- Sample size was small for this species, but its reproductive behavior was very similar to that described for *P. chaudoiri*, including the same four phases of pre-mating struggle, copulation, PCA and post-mating struggle.

In 11 observed mating attempts with females that were resting or walking in the oviposition site, six resulted in copulation and two of these ended with a PCA. One of the 11 couples was attacked by another male; as in *P. chaudoiri* the attacker displaced the riding male and copulated with the female. Four matings with females that were laying eggs were also observed. In two of these matings, the male remained in PCA while the female laid the egg. As in *P. chaudoiri*, females that were in the process of oviposition did not struggle when mounted (Figure 1).

Copulation consisted of a single aedagus insertion lasting 4.1 (±0.7) min (range 3-5 min, N = 7). PCA lasted 7.2 (±7.8) min (range 1-20 min, N = 5). In one of the matings observed, a second copulation occurred after 20 min in PCA. Duration of mounting was not related to male size (R= 0.584, P= 0.30, N = 5).

Egg-laying behavior was also similar to that of *P. chaudoiri*. Females never laid more than four eggs in a single day (Table 1). The oviposition process lasted 13 (±3.8) min (range 8-20 min, N = 9), and 60 percent of observations of females excavating holes culminated in actual egg-laying.

Table 1. Numbers of eggs laid per day by females of two species of *Pseudoxycheila* tiger beetle at a study site in the Andes of Colombia.

Daily number of ovipositions/female	Number of females	
	P. confusa	*P. chaudoiri*
1	5	20
2	1	5
3	0	1
4	3	1

DISCUSSION

Tiger beetles often attempt mating with either males or females of different species, and interespecific hybridization occurs (Pearson 1988). Although *P. chaudoiri* and *P. confusa* were located in the same areas and their reproductive behavior was very similar, we never observed interspecific matings in this study. Our observations support the prediction of reproductive isolation for sympatric species (Cassola 1997), and in this case the species probably are segregated by microclimatic differences (cooler temperatures for *P. confusa*). Tiger beetles are responsive to thermal microenvironments (Schultz 1998) and species segregate by microhabitat preferences (Zerm et al. 2001).

Males and females of both species apparently move frequently among different oviposition sites, because many individuals were recorded in only one visit to the study site. It probably is advantageous for females to disperse their eggs in different sites, as steeply sloping bank habitats are susceptible to disturbance. However, they usually

spent the day at one site and sometimes laid more than one egg there. The mechanism of dispersion to other oviposition zones is likely by flying, perhaps during the evenings or nights as suggested by Cassola (1997). *Pseudoxycheila* spp. are rarely observed flying, but on one occasion NT observed an individual that flew out of sight.

Because *Pseudoxycheila* beetles have poor vision, and there is no evidence of long distance mate-attracting behaviors (Palmer 1976, Cassola 1997; NT, personal observations), males do not locate and pursue their mates from a distance, as occurs in many tiger beetles (Pearson 1988). Instead, *P. chaudoiri* and *P. confusa* males appeared to find females haphazardly when patrolling oviposition zones where fertile females were present.

We did not observe any courting behavior previous to the males mounting the females. Instead, when encountering a female, males immediately attempted mounting them and the females resisted. For two species of *Pseudoxycheila*, Rodriguez (1998) reported a courting behavior consisting of males rubbing the female with his middle legs. The pre-mating struggle exhibited by tiger-beetles may be a mechanism of female mate choice (Pearson 1988).

Ejection of spermatophores during or after copulation has been observed in some species of *Pseudoxycheila* (Rodriguez 1998), and this could reflect cryptic mate choice by females. Attempts by females to dislodge males from their backs could also represent a form of mate choice, by testing the ability of males to hold on (Baena & Eberhard 2007). However, in a sepsid fly, female shaking behavior is a form of communicating her receptivity to the male, and males dismount females that have been recently mounted by another male (Baena & Eberhard 2007).

Alternatively, the pre-mating struggle could simply reflect convenience polyandry (Thornhill & Alckock 1983). Because females in the oviposition areas do not need to mate again to fertilize their eggs, they are reluctant to mate, but males always try to mate whenever they meet a female. Repeated copulations may inflict a mortality cost in females (Blanckenhorn et al. 2002). Thus, females may resist mating but only up to the point that struggling results more expensive than acceptance of superfluous matings. This hypothesis has been confirmed in water striders by increasing male harassment frequency

and showing that females subjected to more harassment become less reticent to mate (Rowe 1992).

As was also observed for *P. tarsalis* (Palmer 1976), in this study males of the two species commonly mated with females that were starting to lay eggs. Mating with females that are ready to oviposit is advantageous for males if last-male sperm-precedence occurs. Males that mate with females at the moment closest to egg-laying avoid sperm competition (Thornhill & Alcock 1983). Even if last-male sperm-precedence does not occur in these species, males that mate with females ready to oviposit usually do not have to deal with a pre-copulatory struggle, increasing the chances of completing copulation.

The absence of a pre-mating struggle behavior in females that are ready to lay, could result if it is cheaper for the female to accept copulation and then resume ovipositing, than to quit laying and struggle with the male, especially if she is likely to lose track during the struggle of the location of the hole she has already dug. However, because females dig in different places before actually laying an egg, suspending the digging process may not necessarily be an expensive activity. Another possible explanation for the lack of reluctance to mate by ovipositing females is that males are better able to affix themselves on the back of the female, making it more difficult (and expensive) for the female to attempt to dislodge them (Palmer 1976).

The post-copulatory association observed in some tiger beetles has been established to be a mate guarding behavior (Kraus & Lederhouse 1982; Shivashankar & Pearson 1994). Guarding the female precludes other males from mating and helps ensure paternity. In our study, however, in most cases the duration of the post-copulatory phase was very short compared with the duration of copulation, and attacks to couples in amplexus were infrequent. In addition, in our sample of three cases attackers always dislodged the guarding male.

Nevertheless, some males that remained in amplexus for a long period (more than 20 minutes) were able to not only guard the female while laying, but also to inseminate her again. Repeated mating can be an important strategy when sperm mixing and competition occur. Thus, the more matings a male obtains, the greater his

chances of fertilizing eggs (Simmons 2001). The post-copulatory association could also be a mechanism for the male to ensure (maybe through a form of mating courtship) that the female will not reject his spermatophore. Apparently, when a *Pseudoxycheila* female rejects a spermathopore, it usually belongs to the last male she mated with (Rodriguez 1999).

Because all matings ended after a struggle initiated by the female, it is clear that females had at least a partial control of the mount duration. The decision to continue in amplexus with the male may be influenced by the status of the female (ovipositing or not ovipositing) and not by male quality (if we take male size as a good predictor of quality).

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