

CHAPTER 6 MATING SYSTEM AND SEXUAL SIZE DIMORPHISM OF THE GREEN ANACONDA (*Eunectes murinus*).

6.1 INTRODUCTION

The amount of energy that each sex invests in reproduction may not necessarily be equal. Males produce smaller gametes than females (anisogamy) and the subsequent investment and behavior of the individual is influenced by this first premise (Bateman, 1948). In general terms, males will benefit most by achieving many matings while the females will benefit most from selective matings with the “best” males. Mating with less fit individuals represents a low cost for males, and a high cost for females. Males are therefore generally polygynous while females are “choosy.” However, if males have few possibilities for obtaining other mates, or if they invest a lot of resources in every mating, it may pay for them to be “choosy” as well (Arnold and Duval 1994; Reynolds and Harvey 1994; Trivers, 1972).

Mating systems can influence other traits in the life history of a species. Sexual size dimorphism has been documented in many species and has been a topic of much controversy. If males and females develop different sizes it is assumed that they are under different selection pressures. Here I summarize the most common benefit and disadvantage that each sex experiences for large and small size applied to snakes. Both sexes benefit from large size because of: 1) increased number and kinds of prey available and the range of prey sizes that it can ingest, 2) deterrence of predators and reduced vulnerability to their attacks (Curio 1976), 3) lower relative energetic costs and higher thermal inertia, which allow them to cope better with temporary shortage of food (Pough 1973). For females in particular, large size benefits in: 1) higher fecundity related to a large coelomic cavity, and 2) the production of large offspring that can potentially have a better chance of survival (Ford and Siegel 1989a; Shine 1994b; Chapter 5). Males benefit from large size because: 1) it may allow them to win mating tournaments and accomplish more matings, and 2) it increases sperm production due to allometric increase in testis size that gives them advantage in sperm competition (Andersson 1994). Large size also can be costly since it involves higher cost of maintenance and increases the risk of starvation in time of prolonged food shortage (Clutton-Brock et al. 1985; Wikelski et al 1997). While larger animals can go without food for a longer time than smaller ones due to their larger reserves, they have larger daily expenses in survival. For instance, male marine iguanas (*Amblyrhynchus cristatus*) are under strong selection pressure for large size due to mating advantages, but each time that the El Niño effect is strong, the largest suffer much higher mortality than the smaller animals due to food shortage (Wikelski et al. 1997). Also, with larger size there may be a higher absolute cost of mobility, which is especially costly in males that have to look for females for mating. In addition, there is a greater detection chance by predators (Shine 1993). Finally, larger size involves longer maturation time (Table 6-1 for a summary; Andersson 1994).

In some species larger males can win more combats, drive away more rivals, and thus monopolize more females (Darwin, 1871; Trivers, 1972). For example, large size enables males to out-compete other males and obtain more mates in mammals (Clutton-Brock 1988), lizards (Dugan, 1982; Rodda, 1992; Stamps, 1983), and snakes (Madsen et al. 1993; Madsen and Shine 1993; Schuett and Gillingham 1989; Shine and Fitzgerald 1995; Weatherhead et al. 1995). In some male lizards, large body size allows forced copulation

with smaller sized females (Dugan, 1982; Rodda 1992). However, in snakes, males are apparently unable to forcibly copulate with the females (Devine 1975; 1984; Shine, 1993).

The underlying causes of Sexual Size Dimorphism (hereafter SSD) in snakes has been a subject of much controversy. Shine (1994a) reviewed sexual dimorphism in snakes related to male-male combat and provided a revised theoretical model. Although males are generally larger compared to conspecific females in those species that have male-male combat, the relationship is not universal. Shine (1978) argued male biased SSD occurs when males fight physically over females and large size enhances the opportunities of the males to mate. Later (Shine 1993; 1994a) he added that the determinant for SSD is the Operational Sex Ratio (OSR). If the probability of encounter of two or more males with a female is low, then male-male combat is not likely to occur. Hence, there is no selection pressure for the evolution of large body size in males. Rather, sexual selection acts to refine abilities to locate the female (scramble competition; Andersson, 1994). Shine (1993) speculated that this should occur when species are in low densities, in aquatic or arboreal habitats, or utilize locomotion methods that do not leave a continuous track, such as side-winding. If there is no selection for large size in males, females biased SSD is a result of natural selection for large female size. There are other species, however, where males fight over the females or obtain more matings if they are larger, and yet females are the larger sex. This has been found in the European adder snake (*Vipera berus* Madsen et al. 1993); European grass snake (*Natrix natrix*; Madsen and Shine 1993b), and Northern water snake (*Nerodia sipedon*; Weatherhead et al. 1995).

The paucity of data on natural mating of snakes, and the poor diversity of taxa studied, compromise our understanding of the issue. Although the knowledge of reproductive ecology of snakes has improved greatly during the last few years (Duvall et al. 1993; Duvall and Schuett 1997; Gillingham 1987; Ford and Holland 1990; 1986; Luiselli 1996; Madsen and Shine 1993a; Seigel and Ford 1987; Shine 1994a; Shine and Fitzgerald 1995), most of these investigations have concerned North American and European natricines and vipers or Australian pythonids. Very little is known about neotropical species (Seigel and Ford, 1987).

Another major gap in our knowledge of snake ecology is the lack of information based on field research (Slip and Shine 1988). Most of the literature concerning snake mating ecology has been performed in captivity, at best with naturalistic enclosures. Published studies of the reproductive ecology of the group are dominated by notes on animals in captivity (Barker et al 1979; Carpenter et al. 1978; Gillingham and Chambers 1982; Hammond, 1988; Perry-Richardson, 1991; Schuett and Schuett, 1995; Tolson, 1983; 1991 among others). Two field studies on the ecology of diamond python, *Morelia spilota* (Shine and Fitzgerald 1995; Slip and Shine 1988) and some studies on the reproductive ecology of the west Indian boas (Tolson, 1992; Tolson and Henderson, 1993) constitute the few references available from field studies, but information about mainland neotropical species is lacking. Long-term field studies are required to understand the evolutionary forces acting on the snakes and their behavioral ecology (Duvall et al. 1993).

The study of life histories and field ethology has been seen as old-fashioned unless focused on answering specific, theory driven questions. However, objective, accurate and descriptive information of what the life of the animal is like is invaluable for an unbiased understanding of a species' ecology and evolutionary history; such observations do not lose validity with every new interpretation of natural phenomena (Greene 1993; Rivas

1997). This research is especially important in secretive species that occur in areas of low human density, such as the green anaconda (*Eunectes murinus*), where information from the field is only anecdotal at best. In this chapter I present field data of the mating system of anacondas and new interpretations in the mating system of other snakes and discuss some of the forces behind it.

6.2 METHODS

Data collection

The field work was carried out during the dry seasons from 1992 to 1998. I did systematic searches by shuffling in the few water bodies that retained water and detecting the animals by feeling them with feet and poles or by visual contact (Chapter 2). In the height of the dry season, I often found masses of several snakes coiling around each other; these turned out to be breeding aggregations composed of a female and several males (hereafter, breeding balls; Figure 6-1). I captured all the animals that were in and within five meters of the breeding balls, processed them, and released them within 24 hours of the capture.

I was able to monitor the development of several breeding balls by force-feeding radio transmitters to 15 females (Chapter 2). Eleven of these females were recaptured after mating was over. These animals were kept in captivity until they gave birth (Chapter 5). I also studied the mating of four females in outdoor enclosures with all the males that were with her in the field. In addition, I monitored the duration of the breeding ball around three unmarked breeding females in the wild by visual observation and, if not evident, searching thoroughly in the places where they were seen. I attempted to film the mating behavior in captivity at the Wildlife Park (WCS, formerly Bronx Zoo) in order to make a thorough description of the mating, but the animals did not make a ball when I tried to film them. Some of the information gathered there is used for the analysis of the findings in the field.

I force-fed radiotransmitters to 28 adult males, to study the duration of males in the breeding aggregations, and to assess male's breeding investment (Chapter 2). I also collected data on duration of courtship from 16 males that I caught more than once in a breeding ball, as well as 3 males held in outdoors enclosures on the ranches where the study was carried out. Nine radio-implanted males were studied after they found a female. Seven of them rejoined the breeding ball after the breeding ball was perturbed. I dropped the two males that did not re-join the ball assuming that the capture might have influenced their behavior. Ten more males were studied by following the radio-implanted females they were courting; while 11 others were re-captured by chance in the breeding ball later. This also allowed me to determine the minimum time they stayed in the aggregation. The data analyses consisted of non-parametric correlations, and mean and variance comparisons performed with SAS 6.10. I did a Levene test to compare the variances (Madansky 1988). I also used the coefficient of variance; which is a statistic that measures the variance in units of the means. Its calculation and statistical inferences are in Sokal and Braumanm (1980; Chapter 3).

6.3 RESULTS

Breeding balls

The first striking finding from examining the composition of the animals caught is the enormous sexual size dimorphism found in the breeding balls (Table 6-2). Each breeding ball was made up of one female and 1 to 13 males (mean 3.83; median = 3; Figure 6-2). The males coil around the female in shallow water forming a mass that usually breaks the surface through the aquatic vegetation. Occasionally, the female lays on the ground at the water's edge, or partially buried in mud. Large females often were found in very shallow water or even on completely dry land. Males remain coiled around the female for many days (see below). In the breeding balls the males coil around the female sometimes covering up her entire body, but usually just the caudal end. In order to provide a description of the mating aggregation, I here summarize serendipitous observations collected in the field before the capture of the breeding balls.

While coiling around her body, the males scratch the female with their spurs, moving them very rapidly in a "tickling fashion". Males also poke the female with their spurs, inducing her to move, and attempting to place their cloaca facing her cloaca. The movement of the spurs occurs in bouts, typically 10 to 30 seconds in duration. Often the female moves or twitches as a response, allowing the male to continue maneuvering his tail under and around the female's tail. The spur movement seems to be an important feature of courtship in anacondas.

When a new male joins a ball he coils around it and starts pushing his way towards the female's cloaca. The other males responded to the arrival of a new male by tightening their coils and attempting to push out the newcomer with their bodies. On several occasions I saw males coiling around the female's neck apparently courting the "wrong end" of the female. On several occasions I also saw some males coiling and "courting" around the tail of a large male, apparently confusing him with the female due to his large size. In one instance a very small female (275 cm TL, 11 Kg) was courted by four males, two of which were relatively large specimens (277 cm TL, 8.75 Kg and 280 cm TL, 8 Kg) I saw these males being courted with spurs and coiled by other males in the breeding ball. In another ball a very large male (310 cm and 11.5 Kg) was courting a very large female (470 cm 68 Kg) with other 9 males of various sizes. I caught them all and put them in a cage to observe the mating. The animals resumed the breeding ball shortly after processing, and mating continued for another 12 days. On one occasion the female moved out of the water and the males hung on to her. Some males, however, were knocked off the female and were scrambling over each other to regain their positions. At that moment the large male who was following the female was clearly mistaken for the female, and several males (sizes ranging from 2.75 Kg to 5.5 Kg) coiled around him performing the spur courtship on his body (Figure 6-3).

In all the events I observed, I never saw any actual or ritualized combat, as described in the literature for other snake species (Gillingham 1987; Gillingham, et al 1983; Tolson 1992). However, the males can only mate if their vent is near the female's, so slow motion wrestling might be happening, where males push each other from the female's cloaca. The mating aggregation does not reveal much movement to the human eye, and it is difficult to appreciate what happens in it. Preliminary analysis of time lapse video taken from a wild caught breeding ball mating in a cage shows that the males are breathing very heavily; by

speeding up the footage the generalized panting of the males is obvious, but it cannot be studied in real time. This panting is not apparent in time lapse footage of single male mating couples staged in captivity at the Bronx zoo. This supports the idea that the animals in the breeding balls are, indeed, struggling physically even though there is no obvious fighting.

On 13 occasions I documented actual copulation during the capture of 11 of these breeding balls (two breeding females were seen mating twice). Hence, courtship and mating do take place within the mating ball. In one instance, I observed actual copulation occurring (i.e. hemipenis inside of the female's cloaca) before I captured them. The copulation lasted for 100 minutes, until dusk, when I had to disturb the animals to collect body measurements. Even at the moment of capture, the male tightened his coils, preventing my hand from getting between his loops. When the resistance of the male was finally overcome the female coiled her tail, gripping the male and preventing him from being removed from his position. Up to this point, the animals were not aware of my presence due to their heads and eyes being under the water and mud. The behavior probably was intended to prevent an arriving male from replacing the mating one. This observation also suggests that females are active participant in mating and not just passively accepting a copulating male.

Breeding season

Breeding balls were observed from mid-February until the end of May (Figure 6-4). Early in the dry season (January, February, and March) it is common to see males actively tongue-flicking, presumably tracking females across different water bodies. It is even possible to find breeding females by radio-tracking searching males (Chapter 2). Searching males move long distances, tongue-flicking thoroughly both the substrate and the air, often crawling on dry land and shallow water (Chapter 3). Larger females were found breeding later in the season than smaller ones as suggested by the modest negative correlation between the size of the female and the date that it was found mating ($r = -0.25$; $p = 0.095$; $n = 46$; Figure 6-5); perhaps a consequence of their larger size that needs more time to gather the heat they need. I was able to monitor the duration of 21 breeding aggregations. The breeding balls lasted for an average of 18 days (range 2- 46; Figure 6-6).

Most breeding balls were captured when first sighted, in order to record data from the individuals. On average, individual males stayed 14 days in the breeding balls; some males stayed with the female until the end of the attractive period (range = 5 - 40; Figure 6-7). I have no information about receptivity of the female to males, so by attractive period I mean the period during which the males are attracted to the females. Again, most animals were found in breeding aggregations and the reported duration probably underestimates the actual time that the each male invests in each female, yielding an OSR of 1:3.83. The sex ratio found in mark and recapture of the entire adult population was nearly even (1:1.22; Chapter 3), so not all the animals in the population are participating in breeding balls every year. Adult female anacondas are longer than males ($t = 15.67$; $p < 0.000$) and heavier in mass ($t = -17.64$; $p < 0.000$). Males showed a smaller variance in sizes than females (Levene test, $F = 71.51$; $p < 0.0001$; Table 6-2; Figure 6-8).

Male tails are shorter (37.44) than female tails (49.06) in absolute size ($t = 5.48$; $df = 195$; $p < 0.000$), due to the large sexual size dimorphism. However, the relative tail length (RTL, tail length/svl) in males is larger than in females (t-test; $t = 7.35$ $p < 0.000$; $df = 195$).

Males have longer and more slender tails than do females. The variance in tail length is smaller for males than for females (Levene test; $F= 11.58$ $p<0.001$). The CV of the tail length is also significantly different between males (0.155) and females (1.021; $t= 28.54$; $df = 223$; $p< 0.000$;) with males showing much smaller variance than females. The RTL of smaller males is significantly larger than the RTL of larger animals (Figure 6-9; $r= -0.388$; $p<0.000$; $n = 177$).

It is also possible to differentiate sex by the size of the spurs, as they are much larger in males than in females (Figure 3-6). Males have absolutely longer spurs (7.47 mm) than the females (5.13 mm; $t= 8.49$; $p<0.000$; $df= 51$) despite the difference in size of the females. The variance in spur length of the sexes did not differ (Levene test; $F= 1.39$ $p< 0.243$) but the coefficient of variation of males (0.784) is significantly smaller than the CV of females (0.98; $t= 2.17$; $p< 0.025$). There was no obvious difference in coloration or pattern between sexes.

Mating System

Due to the long duration of mating aggregations and the fact that they usually take place under water or under vegetation, it was not possible to perform detailed observations of mating in the field. However, I did not observe combat interaction among the males other than pushing. Presumably, the largest male can push other animals from the area around the cloaca, preventing them from mating. Hence, a mating advantage for large animals should be expected. During some of the captures, I managed to keep track of the animals that were mating or around the cloaca in multi-male aggregations. The male coiled around the cloaca was the largest of the group in only 8 of the sixteen balls analyzed. Thus, evidence for advantage for large size is inconclusive. Eight out of sixteen does not seem different than chance for a null hypothesis of 50% probability of occupying that position, but if we consider that the breeding ball is composed by more than 2 (average 3.83) males, then 0.5 might be different than chance. Sadly the variance in the size of the breeding balls and small sample size prevents any simple solution to this problem. In some breeding balls that were in cages, it seemed that the smaller males abandoned the ball first as if they had been excluded from the female; but the fact that they did court a joined the female for several days prevent from drawing any conclusion. In any instance it is apparent at this point that a larger animal does not have certainty of mating and among the determinants of males' mating success, size is only one of the factors.

I documented some instances of several males copulating with one female. I found sperm plugs in 9 captured females that came out during processing. The sperm plugs are made of a friable white mass that produces a strong scent. The mean dimensions from two plugs that looked relatively complete were 7.5 x 1.9 x 0.8 cm; one plug weighted 7.5 gr. (Figure 6-10). Microscopic and pathologic analysis revealed the presence of acellular protein and moderate amount of spermatozoa. These 9 females were being courted at the time I caught them. Thus, the presence of a sperm plug does not prevent females from being courted. In one case studied in captivity, the actual mating was observed. Two days later the sperm plug came loose from the female that was still being courted and coiled by two males (the male that mated included). From another female that was in a cage I collected 5 sperm plugs in the water in a lapse of 12 days.

I did not document any instances of males mating with more than one female. Two males were found in two breeding balls during the same year, but due to our intervention (catching the animals), I cannot be certain that the male would have joined the second ball

without being interrupted the first time. The seven radio-implanted males that were successful in finding females, and that I could follow during the entire period, each found only one female in the whole season. Males appear able to breed every year. Three males were found in breeding balls in consecutive years, while this was never found in females. Females seem to breed every other year or even less often in larger animals (Chapter 5).

I found a positive correlation between female's mass and the average size of the males courting her ($r = 0.38$; $p = 0.010$; $n = 45$; Figure 6-11). This suggests exclusion of smaller males from the breeding balls by larger animals. Larger females were also courted by a larger number of males ($r = 0.48$; $p = 0.001$; $n = 46$; Figure 6-12).

A partial correlation between the proportion of live offspring at birth and the number of males courting the females while holding the effect of the female size constant supports the prediction that females mated with larger number of males have a higher breeding success ($r = 0.51$; $p = 0.027$; $n = 13$; Figure 6-13); which suggests benefit of mating with multiple males. Some of the animals showed a relatively high number of infertile eggs as compared with other animals whose mating was not monitored; this suggests that the very monitoring of the mating might affect the results (Chapter 5).

One of the females studied, Marion (TL= 475 cm; weigh = 70 Kg) was found in an isolated section of river in the only patch of aquatic vegetation in several km of river. I radio-implanted her because I predicted that she was going to breed based on her condition index (Chapter 4) and it would afford a good opportunity to study a breeding ball from the beginning without the disturbance of the capture. I found the animal and made visual contact with her every other day at least, until the time when the savanna flooded. Despite the close monitoring, she was never seen mating or courted by males. However, based on the radio telemetry observations, she behaved as if she was pregnant; she made infrequent movements, moved little and basked frequently (Chapter 3 and 5) which made me decide to catch her shortly before the delivery season. Marion gave birth to 32 stillborn (18 females 14 males; see below) and 16 infertile eggs.

Smaller breeding females were often found covered by mud or aquatic vegetation while large ones were more often found in shallower water or on dry land. Larger females were calm and sedate while smaller ones were more likely to bite and more ready to flee.

6.4 DISCUSSION

Breeding balls and the Operational Sex Ratio

This is the first study of the mating system of any South American boid in the wild and the first contribution to the study of anaconda reproductive biology. Although some reports of anacondas forming aggregations exist in the literature (Blomberg 1956; López 1984) no formal attempt to identify the sex of the animals involved was made; this thus, the first documentation of a mating aggregation of breeding anacondas and the first record of their extraordinary SSD.

The breeding balls show a highly male biased OSR (1:4.2), despite the fact that the adult sex ratio is fairly even. Females make a large reproductive investment, investing on average almost 40% of their post-birth body mass in reproduction; which prevents them from breeding every year (Chapter 5). This male biased OSR increases the intra-sexual selection in males, and the competition for access to the females.

The activity in the breeding ball is very hard to observe since it occurs in the water under vegetation and with several snakes intertwined, which make systematic observations difficult to do. The spur movement seems to be a way to court the female as well as locate the female's cloaca and manipulate her into position for mating. The female clearly reacts to the males poking at her by moving and allowing the males to maneuver with their tails. The difference in size of the spurs between males and females, and the smaller variance in spur size in males, suggest that they are under the effect of selection, perhaps related to mating advantages. The spur movement observed was similar to that found in courtship of other snake species (Carpenter et al. 1978; Charles et al 1985; Gillingham and Chambers 1982; Slip and Shine 1988; Tolson 1980; 1992).

The duration of copulation is variable among the few boids which have been studied. In captive *Epicrates* spp, courtship can last for about two weeks and copulation can last from 30 minutes to nine hours (Tolson, 1992). In *Morelia spilota* mating aggregations can last 4 to 6 weeks (Slip and Shine 1988; Shine and Fitzgerald 1995). Strimple (1996) reports that the mating in captive anacondas can last up to two hours anacondas. Hence, wild anacondas fall within the range reported for other large-sized species. Risk of predation is a limit for mating time and mating aggregations in other smaller-sized snakes. This constraint perhaps does not apply to anacondas due to their large size. The large size of the animals also contributes in decreasing the frequency of eating, since due to their large size the anacondas can endure the long fasting associated with courtship and mating (Chapter 2).

The handling of the water in the llanos might have some influence in the time that the savanna stays flooded and potentially could affect the result found. However, the management does not involve the creation of new habitats but the change in size of the existing; given the large size of the different units it is likely that any effect of the management of the water on the results is only related to the location of the is breeding balls and not any substantial effect on data obtained.

Male-male competition

What is the nature of the competition among the males? I have never noticed any spur combat, body or tail wrestling, or agonistic display in the breeding balls as described in the literature for other snake species (Gillingham, 1987; Carpenter et al 1978; Hammond 1988; Madsen and Shine, 1993a; Shine and Fitzgerald 1995). I was unable to observe the mating with sufficient detail to provide much evidence of male physical competition. However, several males coil around one female, and only the one around the cloaca can mate at the time. This male would be preventing the other males from mating by physically blocking the female's vent. Presumably, a pushing tournament might occur where males exclude each other from the mating position. The generalized panting of the males in the footage suggests that the males were actually physically struggling. This struggle is probably what happened when I tried to separate a copulating pair (see above). Furthermore, the fact that the largest male was in the cloacal position half of the times, even while there were several other males competing with it, suggests that males obtain mating benefit from being large. If there is physical competition for mating, and the largest males have an advantage, sexual selection would benefit the larger males, and large male size would be expected to evolve (Shine 1978a; 1993; 1994a; Shine and Fitzgerald 1995). This scenario does not seem to be supported by the amazing SSD found.

The positive correlation of female size and the average size of the males courting her (Figure 6-11) suggests that smaller males are excluded from the breeding balls with larger females. Therefore, active exclusion of small males by the larger ones is suggested. Another mechanism to explain this trend is by assortative mating, where smaller males court smaller females with whom they have a better chance of breeding successfully. Larger males could be selecting larger females to maximize their breeding effort. Whether it is due to exclusion of smaller males or an ontogenetic switch in mating strategy, the correlation between the size of males and females can be interpreted as further evidence of physical competition among the males. An alternative scenario to explain such correlation is by habitat selection. Smaller males might be limited to search in deeper water covered by aquatic vegetation to prevent predation and thus they are more likely to find smaller females that, for the same reason, use these habitats more than larger ones. This would produce the correlation found without involving physical competition among the males, but it would still mean a larger benefit for larger males, since larger females produce larger clutches; thus selection pressure for large size still exists. This also provides a mechanism to explain the presence of different strategies regarding breeding investment mentioned in Chapter 5.

Breeding balls are relatively common among large snakes. Starin and Burghardt (1992) report seasonal occurrence of groups of African rock python (*Python sebae*) and they speculated that they were mating aggregations. Slip and Shine (1988) reported similar mating aggregations in the carpet python *Morelia spilota* (Slip and Shine 1988), except that in anacondas the animals are all coiled around each other while in *M. spilota* the males may remain in the same areas without apparently much physical contact. This is the reason that some populations of *M. spilota* do not show male combat since the presence of other males involves the risk that a third male might mate while two males are fighting. They in turn mate with the female without combating or guarding (Slip and Shine 1988). However, other populations of the same species do show male combats and do not present aggregations normally (Shine and Fitzgerald 1995). Interestingly, long-term captive anacondas mate without the male coiling around the female (Holmstrom personal communication; Rivas unpublished). In the field however even single-male mating aggregations show the male coiling his tail around the female's tail. Coiling might give the males more physical leverage to hold on their position when another males replace him from the female's cloaca.

Males do not necessarily need to fight over the female to prevent other males from mating. The use of sperm plugs has been reported as a method to prevent other males from mating. Male garter snakes (*Thamnophis sirtalis*) are reported to leave a sperm plug in the female's cloaca which remains for 2 to 4 days, which functions as physical interference to prevent other males from mating (Devine 1975). The sperm plugs apparently also contain "turn off" pheromones that discourage other males (Ross and Crews, 1977). Nevertheless, they have proven to be "surprisingly ineffective" in preventing multiple insemination in garter snake (Schwartz et al., 1989; p: 269). There is no evidence among anacondas of any chemical deterrence for mating, since nine females that were being courted had sperm plugs. In a species with a lengthy breeding period, such as the anaconda, it is unlikely that the plug would absolutely prevent other males from mating mostly due to the short time that the plug stays in the female. Further, considering their strength and constrictor abilities, it is easy to imagine a mechanism by which the male could remove the plug by squeezing close to the cloaca (assuming that the female is neutral).

Males snakes can use complex and diverse reproductive tactics (Madsen, 1993). Male European Adders (*Vipera berus*) induce an uteral contraction (copulatory plug) in the female following mating (Nilson and Andrén, 1982). Male anacondas could be also making a "hugging plug" by wrapping themselves around the female's vent. Thus, there would be an advantage for strong and large animals that could achieve and keep that position. The male anacondas wrapping around the female's cloaca preventing other males to mate resembles the behavior reported by O'Leile et al. (1994) for western diamondback rattlesnakes (*Crotalus atrox*) where the male coils over the much smaller female, covering her with his body and thus preventing other males from having physical access to her.

Due to the metabolic constrains of ectothermy and the presence of many other males, it is unlikely that a male can retain the optimal breeding position throughout the breeding period of the female, regardless of his size. A possible strategy in the mating of male anacondas it that they alternate physical competition and sperm competition by leaving a sperm plug when they can no longer maintain the position. After recovery, males might re-enter the struggle. This scenario would account for the permanency of the males in breeding balls. Even at the height of the dry season females seem to be too dispersed and unpredictable for the male to have a good chance in finding other animals in the same season (see below). Thus, males that mate more often, and prevent other males from mating by maintaining the breeding position for longer and/or by using sperm plugs, would have a higher probability of siring offspring. Future studies need to address in better detail the behavior of males in the breeding ball after mating and what variables determine it.

The male's perspective

If males mated with the first female that they found, I would expect to find no correlation between the traits of the female and the number and size of the males. The positive correlation between size of the females and the number of males courting her; as well as the positive correlation between the average male size with female size, suggests that males actively chose to court larger females. It makes sense for the male to mate with the largest female, since larger females will have both larger clutches and larger offspring. Alternatively this trend could be explained by the female producing more pheromones and manipulating the male's behavior but in any instance it is on his benefit to mate with a larger female since, larger offspring will have higher survival, and, some of them, become into larger females. Hence, mating with larger females produces benefits both in quality and quantity of the offspring (Ford and Seigel 1989a, Madsen and Shine, 1992, 1994; 1997). Choosiness in males is explained by a large breeding investment. A sex would be "choosy" if a mating event forfeits future matings (Trivers 1972).

Are the males performing a large breeding investment per individual female? The sperm plug that was removed from the female's vent represents 0.1% the male's mass. It is fairly high if we consider that the sperm plug is not all the sperm that the male ejaculated and the short time that he has to replenish his sperm reserves within a short mating season. In the llanos the breeding period for anacondas is restricted to the driest part of the dry season (approximately 2 months), the period when it is easiest for males to locate receptive females due to the reduction of the size of water bodies. The time during which males are courting an individual female occupies a significant amount of the breeding season. Such a long time devoted to a female may prevent the males from looking for another receptive female within the same season (Figure 6-7 and Figure 6-4).

One of the males with a radio (Richie, 246 cm TL; 5.8 kg) was found next to a large female (Diega, 401 cm TL; 32.Kg). He followed her for 42 days until the time when she became receptive. During the time that I was following Richie, he passed close by other females that were in breeding condition (Chapter 4). He even passed within 20 meters of an active breeding ball, and did not join it, or courted any of the other females. This strongly suggests that Richie chose to court Diega over the other females; Diega was the largest female that I found in the area. Thus, the long time spent courting an individual female, the large amount of energy spent in sperm, the long time that the males do not feed during courtship (Chapter 2), the assortative mating with larger females, and the individual preference found in individual animals suggests that males are actually selecting quality of their mating over the quantity. The male choosiness advocates against multiple mating in males as an important selection force, since truly polygynous males should maximize the number of mates and not the quality and none of these trends would be present. Furthermore, high mortality of males associated with trailing females or even courting makes philandering less likely (Chapter 5; Rivas et al. 1999; Rivas and Owens In press)

The proximate mechanism for this male choice could be based on: 1) Larger females producing more pheromones and in that way manipulating the male's behavior, 2) Larger females being easier to find, which result in been encountered by more males (Luiselli 1996), 3) Looking for females of a particular size in the areas where they occur most (larger in shallower water, smaller in relatively deeper water), 4) Individual recognition and preference, or 5) Qualitative differences in the scent of larger females a triggering mechanism in the male's *innenwelt* that we are as yet far from understanding. More long term of studies of individually marked animals are needed to fully understand these issues.

The female's perspective

Females would benefit by mating with larger males that would provide genes for large size, which would enhance survival of the offspring and reproductive success of the daughters (Chapter 5). If females select larger males that produce larger daughters, larger (more attractive) sons is a by product of this females preference and an added benefit of mating with a large male. Females would benefit also by siring offspring from males that have high ability to find females, or that have higher courting success.

Female anacondas mate more than one time in one season, and potentially with several males. This is suggested by the fact that several females that had sperm plugs, were also being courted. The long duration of the breeding ball and the strongly biased OSR creates opportunities for multiple mating (Barry et al. 1992). Multiple mating and insemination have been reported in several species of snakes (Stille et al., 1986; Slip and Shine, 1988; Schwartz et al., 1989; Barry et al., 1992; Höggren and Tegelström, 1995; McCracken et al. in press).

There has been some discussion about the benefits that a female receives from multiple matings. Slip and Shine (1988) argue that females mate multiply to ensure that they obtain sufficient sperm to fertilize all the eggs, and to reduce the cost of rejecting males. Indeed, some females I caught and interrupted during mating had larger number of infertile eggs when they gave birth than unperturbed females so several matings might be required. Multiple matings might be needed to provide enough sperm to fertilize all the eggs or to provide high diversity of sperm; but this mating need not be with different males since several single-mated females had normal clutches. Further, in captivity anacondas breed regularly with only one male (Holmstrom personal communication; Strimple personal

communication). Loman et al. (1988) argue that multiple insemination would guard against the possibility of genetic defects and by increasing the genetic diversity of the brood in unpredictable environments, where the female cannot assess the genetic quality of the male. Offspring diversity would also decrease the competition among siblings. Zeh and Zeh (1996) argued that the use of diverse sperm is a hedge against genetic incompatibility and conflict at the suborganismal level acting among intragenomic units (see also Jennions 1997). In a system where sperm competition is important then multiple mated females have offspring from spermatically competitive males produces a Fisherian phenomena selecting for males with highly competitive sperm (Keller and Reeve 1995). Madsen et al. (1992) found that multiple mating in *Vipera berus* results in higher proportion of ova being fertilized by males of higher genetic quality, by means of sperm competition. Olsson et al. (1994) showed similar results for *Lacerta agilis*. Seigel and Ford (in press) found that female *Lampropeltis triangulum campbelli* show increased fecundity when mated with several males as opposed to females mated multiply to the same male. My findings suggest that the females benefit also from multiple matings as suggested by the relationship among the number of males in the breeding ball and the higher success of females courted by multiple males.

Females are not expected to engage in multiple matings when a mating event increases the risk of injury or death during or after the mating (Arnqvist, 1989). In anacondas, however, the large size of females poses little risk while mating. This is consistent with the sedate behavior exhibited by large breeding animals and by the increased exposure by larger females when basking and laying on the dry land or very shallow mud. If females mate with many males, then sperm competition should be at work. Multiple matings have been argued to decrease the benefit to the males of being larger since the benefit that a large male might have of mating with a female will be diluted by the fact that other males might mate as well, and the competition may be at the spermatic level. However, larger males can benefit by having larger testes that produce more sperm. This has been found to be the case in pythons where larger males have relatively larger testes than smaller males (Shine et al. 1999a; Shine 1999b).

Madsen and Shine (1997) reported that, based on recapture of marked clutches, the survival of the clutch was highly determined by its quality and not its quantity. They thus concluded that having high quality offspring was more important than quantity in the female's fitness. If so, selecting for good mates maybe a critical trait in the mating for the females. Female choice has been reported as an important issue in the mating system of snakes (Barry et al, 1992). Male snakes are apparently unable to forcibly copulate with females due to the elongate shape of the body (Shine, 1993). Furthermore, a snake's hemipenis is not designed for forcible penetration by thrusting (Devine, 1975; 1984). For instance, in the genus *Epicrates* copulation cannot be accomplished if the female does not open the cloaca and allows intromission (Tolson, 1992); and a similar phenomenon seems to occur in other species such as *Agkistrodon contortix* (Schuett and Gillingham, 1988) and *Crotalus atrox* (Gillingham et al., 1983). Females are known to be highly selective in mating aggregations; Perry-Richardson et al. (1990) found that female *Thamnophis marcianus* reject some males, even after intromission has occurred. While breeding several generations of *Thamnophis melanogaster* in the laboratory, it has been also noted that sometimes females accept some males and not others (Burghardt, personal communication). Joy and Crews (1988) suggested some individual males may be consistently more successful than others. Female choice may be even more important in

robust constrictor species where the females are much larger than the males. My observation of female behavior preventing the male from being removed from his position, suggests that the female does take an important part in the process.

The female can also use other ways to control the paternity of their offspring. Eberhard (1996) reviews the many ways that the female can exert control over the paternity of her clutch using post-copulatory mechanisms; from discarding the sperms of some males to abortion of clutches that are doomed to be killed by a new male in the group. Selective abortion of clutches that had an undesirable sex ratio has been reported in coypu (*Myocastor coypus*) (Gosling 1986). Selective development of some eggs is not among the possibilities presented in the literature, perhaps for lack of significant field evidence that suggest this possibility. The undeveloped fertilized eggs found in one of the females suggests the possibility that the female might be able to develop some individual eggs and stunt or prevent the development of some others. That the female selectively produces the death of some individuals in the womb is an interesting hypothesis that deserves to be studied (Chapter 5).

What are the proximate mechanisms by which the female selects her mates? Females that start signaling earlier in the season will have the benefit of having more males available since few are already committed in breeding balls. This would provide the female with many males to choose from and would encourage competition among them; therefore, by means of signaling prematurely she selects for larger males that can withstand longer duration of competition with other males; while she also gives more time for the largest males to establish themselves.

The analysis of RCM indicates that larger females incur a smaller investment than smaller females. However, RCM might not be the right currency to measure the breeding investment. Larger females are at a greater risk when feeding or hunting and incur a larger risk of death (Chapter 3). Another approach to measure the reproductive investment could be considering the time that the female needs to gather the energy surplus for reproduction since it would compensate for the different scenarios that the animal has to face. This other approach shows that smaller females invest in reproduction the surplus gathered during the last two years of foraging, while the larger females invest the surplus gathered in three or four years. This approach predicts that larger females should be choosier than smaller ones, since siring offspring from unfit males would cost so many years of mortality risk until the next breeding event; while a smaller female only requires two years to breed again. However, to make inferences about the choosiness of the animals is premature until more field data have been gathered and mating preferences are better understood.

Thus it appear that larger males would have an advantage in their ability to out-compete other males in the aggregation by being able to search for females in different habitats with less risk of predation, by having larger testes that gives him an advantage in sperm competition, and by being selected by the female to provide genes for large size for her daughter. Furthermore, homologous morphological traits in males and females are expected to show high genetic correlation (Halliday and Arnold 1987); hence any selection for large size in females should also increase the size of the males. All of these are advantage for large size in males. Males would, of course, suffer some disadvantage of being too large (summarized in Table 6-1), but these benefits seem to out weight the selection pressures for small size summarized.

Sexual Size Dimorphism

Anacondas have the largest SSD reported in any tetrapod. The largest female I found was 97.5 kg: 40 times heavier than the smallest adult male found (Table 3-1; Table 6-2). This is a SSD without precedent among terrestrial vertebrates; it is rivaled only by the southern elephant seal, *Mirounga leonina* (Bryden 1972). The average female anaconda is 4.7 times heavier than the average male size, which is larger than the SSD found in most other species of vertebrates. Large SSD can be explained by a) selection for very large size in females, and/or b) selection for very small size in males. I have already discussed the selection for large size in females (Chapter 5). In the rest of this section I will discuss other selection pressures for male size.

The relationship between male-male combat and male size has been discussed broadly (Shine, 1978a; Madsen et al., 1993; Shine, 1993; Madsen and Shine, 1994; Shine and Fitzgerald 1995). These authors argue that large size in males would increase their likelihood of winning combats, and the winners would have a higher probability of breeding. Conversely, a lack of selection pressure for large body size would produce small size in males (Semlitsch and Whitfield-Gibbons, 1982). Unlike other mating aggregations, in anacondas the males are permanently coiling around the female and any struggle would be *over* the female, so the male that positions himself around the cloaca is the only one that has the opportunity to mate. It would benefit the male to stay there for as long a time as possible, either by actually mating, or by preventing other males from subsequent matings (contact guarding; Parker, 1984). Presumably females would benefit for mating with larger males (see above) thus female choice may also favor larger sized-males.

Smaller adult size of males can be explained by increased mobility, which may offer an advantage in terms of locating females and moving on dry land or very shallow water, less conspicuousness, and less energetic requirements (for a revision of the advantages and disadvantages of large size). Other than the advantage for small size summarized in Table 6-1, it can be argued that smaller male size may also be a consequence of early maturation (Weatherhead et al. 1995, Madsen and Shine 1993c). Younger males benefit from early reproduction because their fecundity is not dependent on size and breeding early allows them to secure reproduction. Even in those species where male combats occur and the selection for large size is present, some smaller males may have some mating success in some years due to fluctuation in the OSR (Madsen and Shine 1993c). The reproductive investment of the males may be relatively low and they do not forfeit much growth by breeding early (Bell 1980); so males would benefit from maturing early as this would give them some chance of breeding (Madsen and Shine 1993c; Weatherhead et al 1995). Female biased SSD is the most common scenario among snakes (Shine 1994b) and may be the ancestral condition among of the group. Phylogenetic inertia would explain the female biased SSD if there was not a strong selection pressure for large size in males. In our case with anacondas, the magnitude of the SSD is such that one must look for other reasons. Furthermore, this explanation alone does not account for the difference in variance found between both sexes. The smaller variance in the male size suggests that they are under a stabilizing selection toward an optimum size (see below).

The possibility of cannibalism is another important element that might influence the SSD. Presumably larger males would be in better condition to prevent predation by the female so the males might benefit from being large. However, the size difference between males and females is such that an increase in male size might not be enough to save him.

His size might be constrained for the other reasons (mobility, early maturation etc). Therefore, a male might benefit also from not being too good a meal for the female, and by maintaining an unworthy size that may not be profitable for the female (Chapter 5)

Optimal male size: wearing the snake's shoes

The dynamics of the breeding ball suggests that there are both some disadvantages and advantages for large size in males. It is possible that the male's size represents the equilibrium between the interaction of several selection forces. There could be other forces leading to small size that would balance them out producing a local optimum.

Rivas and Burghardt (in prep.) proposed that in breeding aggregations the size of the female could be a sex recognition cue for males in the breeding ball by use of tail contact, since eyes and chemosensory organs are facing away. This sets the limit for the maximum size of males at the size of the smallest females, since being mistaken for a female might make the male lose time and effort needed for courting and mating. To seek and court large, bulkier animals makes sense for a male, since larger and thicker females have more offspring (Ford and Seigel 1989a; Chapter 5), are more likely to be breeding (Chapter 4), and they are also older and more experienced. Hence, it would benefit males to court the animals with largest girth, both for certainty of courting the right sex (and individual) and for increase of fitness. Thus, SSD could be the key for sex identification in situations where the chemosensory organs are not involved, or the pheromones of the females and scents of the males have impregnated all of the animals in the seething breeding ball. Success may belong to the male who can best discriminate males from females, maneuver into position for copulation, and thwart other males from doing likewise.

Confusion of large males with females in the breeding ball seems to be the case in those events where larger males were coiled and courted by smaller males (Figure 6-3). This confusion seems to have been the case also in the experiments of Madsen and Shine (1993b) with grass snakes (*Natrix natrix*), where they report that "Males seemed to become confused between the female's tail and those of other males, and the tails of rival males often become entwined" (p: 562). This also appears in the findings by Noble (1937) where he reports that two small male *Thamnophis sirtalis* courted, for half hour, a large male from another region where the animals were not reproductively active at the time. The hypothesis of SSD as a cue for sex recognition in the breeding ball seems to be supported by fact that the overlap of sizes of males and females is very small (Figure 6-8). Indeed it is possible to accurately assign a sex to an adult individual given only the size and length. If there is a complex of evolutionary forces leading to a "optimal size" in males where they are larger enough to win combat but not too large to be mistaken for a female, stabilizing selection is at work. The size range of males is much narrower than the size range of females (Figure 6-8; Table 6-2) which supports this idea; the significant difference found in the variance and coefficient of variations of males and females also supports this hypothesis. Larger variance in females could be a product of different ages with older snakes being larger than younger ones. The smaller variance in males, however, can be explained by directional selection resulting of selection toward large size to escape predators and perhaps mating advantage in the breeding ball, and a limit of maximum size not to be mistaken for a female. This confusion in males in the breeding balls may not be exclusive to anacondas and a detailed analyses of the relations among the variances females and male size in other species of snakes that form breeding aggregations could shed light on the generality of this phenomena.

In some species SSD occurs at birth, where typically males are born smaller than females (Weatherhead et al 1995; King 1997). This does not seem to be the case in anacondas where the size at birth is not different among the sexes in length or mass (Chapter 5). An explanation for the mechanism of the strong SSD and small variance of male size found is that males grow fast (like females), until they reach the optimum size where they stop. Fast growth rate allows males to escape predation and enter the breeding arena earlier, and stop the growth at some point where it is convenient to keep the optimal size for breeding. This is supported for the mark and recapture data where many males did not experience any perceptible growth in as many as six years (Chapter 3), however but a better analysis of the population dynamics is needed.

Females experience a switch in diet after reaching about 3 meters in length. They switch towards more energy rich prey items (non-avian prey). This switch in diet from birds to mammals and reptiles might be related to the increase in energy that allows them to grow larger (Chapter 5). Thus a possible proximate mechanism by which males maintain the smaller size is by maintaining a lean bird-based diet and a relatively low feeding frequency. In fact a captive-born male at the Bronx zoo, fed *ad libitum* with mammalian prey developed an exceptional mass of 40 kg which is remarkably higher than the size attained by any of the wild individuals. Sadly, the origin of the animal is unclear since they come from the pet trade or donations that the zoo receive.

Differential maturation among the sexes may be involved in the evolution of this mechanism of sex identification. Females delay maturation since they make a larger breeding effort. Males start breeding earlier and at a smaller size than females; since females need to gather more energy to start breeding and the fecundity independent cost of reproduction is too high to produce a small clutch (Bell 1980; Madsen and Shine 1994). This differential maturation sets the scenario for natural selection to act and SSD can be selected as a method of sex discrimination. Once the females are larger and thicker the stage is set for natural selection to target it as a mechanism of sex discrimination. The idea that natural selection can act on pre-existing traits and use them for complex communication systems has been discussed broadly in the literature. In empid flies, behaviors with an original ecological significance have been incorporated into the ritual of mating and have taken on a different role than they use to (Kessel 1955).

Looking at the female perspective, what decision process is taking place in the female? In a breeding ball the females are courted by several males at the same time. It is very likely that the only way she can discriminate and choose among the males is, again, by relying on tactile cues. Does she have the ability to differentiate from the displays given by the head of the snake in her dorsum or neck, which tail is worthy of her favors? It may be necessary to observe a mating ball three dimensionally from the interior to fully understand the processes involved; using genetic markers to determine which males are successful is only a first step in the analysis needed.

Mating system

It remains to be explained why breeding balls last for so long. Lengthy mating period involves larger exposure to predators, reduces foraging efficiency, and in males, it forfeits other matings. Here I identify several possible explanations: First, the female is randomly receptive throughout the period, and mates with different males without much discrimination; in which case she is simply encouraging sperm competition among the males (Schuett 1992; Madsen et al 1992; Westneat 1996). Second, she is not initially

receptive to the males but puts out pheromone and attracts several males that would compete physically over her; by being receptive only at the end of the period she would be selecting the stronger males that have endured the struggle. Third, females lay in shallow mud making a breeding ball that is conspicuous to predators; she is safe due to her larger size but the smaller males are at risk of predation; thus the handicap principle is at work (Zahavi 1975). Fourth, she entices courtship that allows her to select the best males in the ball by mating selectively with the most appropriate using some criteria unknown to us (perhaps assessing some aspects of the courtship related to the spur movement). It is not clear at this point what the situation is and future studies should address these issues.

In species where the female mates with many males, sperm competition is at work (Parker 1970). Recent models (Westneat 1996) argue that males who mate last have greater benefits for several reasons. First, passive sperm transfer from one compartment to another in the females reproductive tract; so there are fewer sperm that have been lost from the last ejaculation. Second, the displacement of sperm from one ejaculation by another, the later male would flush the ejaculate of former males giving advantage to the later males. Third, stratification of sperm in the compartments of the female's system gives advantage to the last male to mate since when the sperm is going to be used the one that was deposited last is more likely to get to the ovules (Westneat 1996). Much controversy still remains in this issue (Wirtz 1997) but the last mate advantage model may explain the large time the males spend in the breeding aggregation.

Females anacondas are capable of sexual cannibalism (Chapter 3; Rivas and Owens, In press) or simple cannibalism (O'Shea 1994). This imposes a dilemma for the males. The longer he stays with the female the more likely he will sire her offspring and prevent others from doing so; but also the higher the risk that he might be eaten by the female at the end of the breeding period. This selects for refined abilities of the males to detect the mood of the female, and an abrupt dissolution of the ball as opposed to a gradual one. The latter hypothesis is not supported by some field observations.

Another factor to consider for the long duration of the breeding balls is the low probability of encountering other females. Females are not clumped together and have a rather unpredictable distribution; thus males looking for females must travel relatively long distances during which they must face high risk of predation. It is possible that it does not pay for the males to leave the ball and try to find another female. It is a testable hypothesis that they are better off by trying to secure some offspring from one female once they find one. Duvall et al. (1992) argue that if the chances of the male of finding a female are low the male should improve his convincing abilities once he finds one female. Shine and Fitzgerald (1995) found that males were more insistent in courting a female in those areas where the density of animals (and thus the encounter rate) was lower.

Breeding occurs in the dry season, when the availability of aquatic habitats is low and presumably females are relatively easier to find. Due to the reduction in water level, the encounter rate between males and females increases, and the males are more likely to find the females (Shine 1993; Duvall et al 1992). The breeding season ends as soon as the rains flood the savanna and the anacondas start dispersing. The time that a female is attractive to males is remarkably long compared to the length of the mating season. It is, therefore, unlikely that a male would have enough time to track, court, and mate with another female in the same season. So, males in general might have time to court only one female in a season. Thus, a male biased OSR (driven by large RCM of the females), a short mating

season, a long receptive period (perhaps driven by female choice), and high unpredictability of the breeding females, along with relatively high predation risk for males in searching for females, might be responsible for the first instance of a polyandrous mating system in snakes.

Duvall et al. (1993; 1992) proposed a model for the evolution of mating systems that predicts the mating system expected based on the female spatial predictability and female temporal availability. Several types of polygyny can evolve from Female Defense Polygyny (FDP), Prolonged mate search polygyny (PMSP), Explosive mating assemblage (EMA), and hotspot polygyny (HS). They also speculate about the possibility of resource defense polygyny (RSP) as it is present in other taxa. They argue that polyandry is constrained in snakes due to phylogenetic reasons. Anacondas do not fit into any of the patterns described by Duval et al. (1992). The anacondas' mating system in the llanos would be in the corner of the axes with low spatial predictability and lengthy receptive period under a label such as Lasting Breeding Balls Polyandry (LBBP; Figure 6-14)

The lack of the potential for the evolution of this mating system in Duvall's model is understandable due to their lack of any appropriate field data, and the fact that most studies have been conducted on a few taxa (Natricines and Viperids). This lack of geographic and systematic representation in previous studies of snakes mating system did not offer a sufficient gamut of possible mating systems in which to build a model. A better understanding of the dynamics of the breeding of snakes awaits more diversified information on the mating systems based on field studies.

Fisher's Runaway process

Fisher's runaway process has been used to explain the evolution of exaggerated traits in a species that confer advantage in mate choice. Particularly the development of antlers, size, or other features beyond that dictated by natural selection. Sexual size dimorphism is one of these traits that can develop under a runaway process. Females select large-sized males thus most offspring are sired by a few very large animals and this preference for large males is transmitted to the female's daughters. If females prefer to mate with large males, this locks up the population in an ever increasing positive feed back loop where male's large size grows beyond the required for the survival of the individual (Fisher 1930).

Anacondas and other groups where extreme SSD is found, such as marine mammals share several common features. Such features include: breeding aggregations that suffer relatively low predation pressure, aquatic environment, and general large size; the latter is often correlated with large SSD. It has been proposed that large size marine mammals relaxes several ecological constraints such as predation pressure, competition with other species, and metabolic efficiency, allowing expression in full of the sexually selected characters (Andersson 1994; Lande 1980). Is it possible that an equivalent situation is happening in anacondas leading to the evolution of large size in females? In marine mammals the largest sex clearly obtains a benefit from mating with several females and siring that many more offspring (Le Boeuf and Reiter 1988). Is there in equivalent process in female anacondas?

Female anacondas have a very large variance in their breeding success, and large females are selected more by choosy males. The preference of males for larger females

increases the survival of her clutch, and potentially the fitness of it as well. Thus, male preference increases the female's reproductive output. These are the characteristics that increase the sexual selection gradient in a population (Duvall et al. 1992; 1993; Arnold and Duvall 1994; Wade and Arnold 1980). Thus, it is a testable hypothesis that sexual selection acts by increasing body size in females. The large size of the female can also be a consequence natural selection (Chapter 5) as well as sexual selection favoring large dimensions ("a sexy daughter"). This should be another variable to include in Figure 5-26 and could also explain further the presence of females larger than the expected in the model represented on Figure 5-25. Hence, stabilizing selection acting on males for an optimum size, and sexual selection (runaway), along with directional natural selection acting on large size in females would explain the extreme SSD found.

6.5 GENERAL DISCUSSION

Sexual Size Dimorphism in flooded habitats

In Chapter 5, I argued that the situations where I studied the animals are peculiar due to the hyper-seasonal savanna and I speculated what the differences would be in a different habitats such as rivers or deeper environments where the anaconda is likely to have evolved. The llanos is a very recent ecosystem (López-Hernández 1995) and the anacondas probably evolved in the neighboring rain forest or in the ancestral llanos that was some sort of interior sea, perhaps with similar seasonality but more flooded in general terms (Rivero-Blanco and Dixon 1979). In this section I will speculate what the mating system would be of anacondas in other habitats.

Flooded habitats would bring two major differences to the biology of the snakes. One of them is a decrease in the encounter rate among the animals. The rate of encounter between males and females, males and males, and anacondas with other species both prey and predators would be decreased. Deeper water would also involve a lower availability of small prey items (wading birds and fowl) so the diet of the anacondas might be more unpredictable (Chapter 5). The decrease in the encounter rate with prey and the presence of larger and more unpredictable prey would select for the development of larger size, since larger animals can withstand fasting for longer periods. Predation on smaller animals might be decreased due to the lack of encounter with predators so that the pressure for large size to fend off predation in the llanos might be relaxed, but the limitation for lack of small prey may produce a new pressure for large size.

The lower encounter rate between males and females might produce changes in the mating system. If the males find females less often the breeding balls that I found might be less common or might not happen at all. The physical competition among the males might not be as important. Instead high mobility and refined senses to track the females would be selected for. High mobility tends to select for small size since it lowers the energetic costs of moving; however, in water the expenses of locomotion might not be too high either, and this limitation may be relaxed. If the mating balls still occur then selection pressure for large size will still be present. I argued that the females in the river should reach larger sizes (Chapter 5) so the optimal size for a male may be pushed upwards. The SSD in rivers might be the same as we find in the savanna, since both males and females may be larger, but there are so many variables involved that it is difficult to make predictions. Recent studies in mating systems of snakes show an amazing variability of morphs within the same species where one population shows high SSD and no male

combats, while another population in a different geographic regions shows violent male combats and no breeding aggregations (Shine and Fitzgerald 1995). At the Bronx Zoo (New York) anacondas mate without the males coiling around the female when they were in pairs (Holmstrom personal communication) and when there were several males as well (Rivas unpublished). However in wild caught single-male breeding balls the males do coil around the female. Thus variability in the males courting behavior of anacondas is also present and deserves to be studied.

Breeding frequency

I showed how the breeding frequency of the female decreases with age and I argued that in rivers this decrease might be less steep since feeding rates might be higher; alternatively the prey might be more profitable or less risky. However, what would happen if females are fat enough to breed but less likely to be found by males in the lower density of the flooded riparian habitats during the mating season? When the females invest such a large amount of energy and effort in each breeding event, it is very important not to breed without sperm provisioning of the male. It is possible that ovulation does not even start until she is mated, or at least courted in order to ensure that her eggs are going to be fertilized. If there is no male suitor the female is better off by saving the energy for next year when she may be found by males. It has been found that some parts of the female's ovulation may be induced by the courtship, or some seminal substances that the male provides along with the sperm (Eberhard and Cordero 1997; Whittier and Crews 1989; Whittier et al. 1987). However, the female might benefit from breeding whenever she can due to the risk of dying during a feeding event or other causes of mortality; since the influence of CI on the clutch size is not as important as the influence of length (Figure 5-8). If the possibility exists that the males may not be available at the time when the female is ready to breed, she might resort to sperm storage for future reproductive events when she has it available. Long term sperm storage and even facultative parthenogenesis has been reported in several taxa of snakes (Schuett 1992; Schuett et al 1997). Long term sperm storage also might be what happened with Marion who gave birth in an isolated piece of a small river without forming a ball and almost certainly without mating that year. The fact that the offspring were males and females suggest that it was long term sperm storage and not facultative parthenogenesis since the latter produces only males (Schuett et al 1997). The null reproductive success indicates that this way of breeding is not the preferred method and the females is better off at being courted and having a large and perhaps diverse provisioning of sperm to chose from. The sperm that she stored was sperm that she did not use in her last reproduction and was presumably not from the "preferred" male(s).

Polygyny in snakes

Even though multiple insemination has been documented before (Barry et al. 1992; Gibson and Falls 1975; Höggren and Tegelström 1995; Madsen and Shine 1993; McCracken et al. in press; Schwartz et al. 1989; Shine and Fitzgerald 1995; Stille et al. 1986), this is the first report of polyandry in any snake species. The closest that some authors have come to acknowledging polyandry is by using the word "promiscuity" (Shine and Fitzgerald 1995), but no further discussion is provided to it and all of their findings were analyzed in the light of "female defense polygyny" or "mate-searching polygyny" (p.: 496).

Up to the present all the reports of mating systems of snakes regard them as polygynous (Duvall et al 1992; 1993; Arnold and Duvall 1994; Duvall and Schuett 1997; Shine and Fitzgerald 1995). However, multiple matings by males per breeding season has been largely assumed and seldom documented (Gibson and Falls 1975, Schuett 1982). Often a male courting multiple females is considered as evidence of multiple mating in males without confirming whether those courtships were successful or artifacts of captivity (Weatherhead et al 1995; Shine and Fitzgerald 1995). Furthermore, multiple copulation by one male is not really enough to warrant polygyny, since some matings might not produce any offspring.

Evidence for multiple mating in males is limited to few studies. Blanchard and Blanchard (1942) documented multiple mating of one male *Thamnophis sirtalis sirtalis* but all their observations were on captive animals. Madsen et al (1993) provides perhaps the clearest example of a species where males European adder were found mate multiple times in a season on a regular basis. About half of the males mated with more than one female and about 2/3 of the males accomplish some mating; plus all the females mated with multiple males as well. This does not really resemble the situation of typical polygyny where few males do most of the matings and most males are relegated to bachelorhood (Clutton-Brock et al. 1988; Le Boeuf and Reiter 1988). The high variance in males mating success is the key for sexual selection to exist (Bateman 1948) and a runaway process to occur, and this is not present in the European adders.

The anaconda polyandry might not be just a rare exception to the Duvall et al. (1993) models. True polygyny might not actually be nearly as common in snakes as formerly believed. Several features of the mating system of snakes suggest this. First, the large investment that some males make in single reproductive events (Weatherhead et al. 1995; Madsen and Shine 1993b; O'Leile et al 1994) which lower the likelihood that the males mate with many females. Second, the OSR of many species is male biased (Weatherhead et al. 1995, Slip and Shine 1988; Madsen et al. 1993, Madsen and Shine 1993a, Luiselli 1996) because the females make a very large reproductive investment and they cannot afford to reproduce every year (Madsen and Shine 1993a) creating great potential for the female to mate multiply (Barry et al 1992). Third, females have large variance in reproductive success associated with her size (Weatherhead et al. 1995, Shine 1994b; Ford and Seigel 1989a) which may lead to (fourth) male choosiness in selecting the best female in several species (Luiselli 1996; Garstka et al. 1982) and to an increase in the intensity of sexual selection in females (Wade and Arnold 1980). Finally, the most convincing argument that the dominant mating system in snakes is not polygyny is the very fact that multiple paternity has been found in nearly all the species where it has been studied in detail (Barry et al. 1992; Schwartz et al. 1989; Gibson and Falls 1975; Höggren and Tegelström 1995 Madsen et al. 1992; McCracken et al In press)

There has been great reluctance among researchers to consider that mating in snakes is not polygynous. Duvall and Schuett (1997) found that only 12 of the 28 radio implanted males *Crotalus viridis viridis* were successful in finding females, while 17 of the 21 females studied were courted and presumably mated. Only four of the males courted (and presumably mated) more than one female (9 matings in total), while 12 of the females mated with multiple males (32 mating in total). Notice that the proportion of males that mated with several females (33%) is much lower than the proportion of females that mated with several males (71%). Notice also that the average male that mated did so with 1.4 females while the average female that mated did so with 2.7 males. Surprisingly, the

authors insisted in labeling this mating system as a polygynous one (p. 333) despite of the fact that it is clear that multiple mating in males is not the norm and its importance as a trend in the evolutionary scenario of the species is not certain.

Failure to identify polyandry (or at least lack of polygyny) has led to a very long lasting controversy about female biased SSD in species where males fight physically over the females. Madsen and Shine (1993*b*) argue that if females obtain more benefit by being large, then they could be larger than males despite the mating advantages of larger males. In a polygynous mating system however, the benefit of the female of being larger (natural selection) could not be higher than the benefits of males of being large and hence mating with more and larger females (sexual selection). Indeed the sexual selection gradient for the males increases with the female fecundity (Duvall et al 1993; Arnold and Duvall 1994). In other words any increase in fecundity of the female for being large will be followed by a equivalently geometric increase in the male's reproductive output by mating with several of those females with enhanced fecundity. Thus, the explanation of Madsen and Shine (1993*b*) cannot really account for the permanence of female biased SSD in species where larger males have mating advantages unless one considers that the mating system is not really polygynous.

Sexual selection acting on females

Even in the cases where females are documented to mate multiply and get benefits from multiple mating, the authors have not considered the influence of this particular mating system on the females life history and how it affects the sexual selection gradient and intensity of sexual selection on them. Multiple mating has been considered in regards to how it affects mating success and life history traits of the males, and SSD considering only the male side (Shine and Fitzgerald 1995; Slip and Shine 1988; Madsen et al. 1992; Olsson et al 1996; Madsen and Shine 1993*c*; Madsen and Shine 1992) but no consideration has been given to the effect of it on the female's reproductive biology.

A high sexual selection gradient (SSG) can produce several trends in the mating system of the females that one can identify. To gather this information from the published literature is fairly challenging, since it has never been the aim of the papers and the information is scattered among several taxa. However, it can prompt different analyses that change our understanding of snake ecology and evolution. First, female's reproductive success can increase by accomplishing multiple mating (Madsen et al 1992; Seigel and Ford in press; Olsson et al 1994; this contribution). Second, the large size of the female increases mating success via male choice (Luiselli 1996, Garstka et al 1982; this contribution). Third, larger females have more and/or larger offspring (Ford and Seigel 1989*a*, Shine 1994*b*; Chapter 5). This means that there is some variance in the reproductive success of the females depending on their size; this sets the scenario for sexual selection on female size. It actually might be the case that anacondas meet all of the above characteristics in a more dramatic way than any of the other species or that its larger size allows the full expression of SS.

The difficulty that snake researchers have had in actually having valid data on snakes mating systems has led to much controversy. Perhaps misled by the voluminous sexual selection literature on organisms that do not grow much after adulthood (mammals, birds, and insects) and their theoretical "dogmas", they have overlooked many aspects of snake biology despite the suggestive data they had in hand. This is, perhaps, an undesirable consequence of the male bias among snake biologists (Wilson 1998) that might have

prevented the snake biologist from considering the female perspective. Such bias among scientists influencing the interpretation of the biological data has been documented before (Cunningham and Birkhead 1997; Gowaty 1994). To record naively the natural system without any bias or the intention of predicting it has great potential to understand better the natural world by allowing a higher diversity of hypothesis testing in the conceptual framework (Rivas 1997). As argued elsewhere (Rivas and Burghardt in prep), through critical anthropomorphism and consideration of a species' or sexes' *Umwelt*, such research bias can be reduced.

Snake's evolution and low encounter rate

To assume polygyny as the general mating system of snakes may appear sensible due to the fact it is the most common mating system in the group that the snakes apparently derived from Thecoglossian lizards (Forstner et al. 1995; Lee 1997;). However, there are several traits of snakes as a group that are different from other squamate reptiles and that can be related to the lack of polygyny. Snake lack the territoriality and the male biased SSD that is the general trend on their ancestors (Phillips 1995, Wikramanayake and Dryden 1988; Stamp 1983; Shine 1994a). There is yet another important difference between snakes and varanids and it is related to the reproductive investment. Snakes tend to have much larger reproductive investment than lizards (Siegel and Fitch 1984). I believe that an evolutionary environment with a low encounter rate is a likely scenario of the evolution of snakes as a group and could explain the evolution of all these synapomorphies.

There is some controversy regarding the origin of snakes. The latest evidence suggests that the ancestor of snakes was from some maritime origin (Caldwell and Lee 1997) and the more derived groups evolved in some fossorial habitat (Forstner et al 1995; Gans 1975; Lee 1997; Rieppel 1988). In any case the encounter rate might have been low. In a fossorial environment mobility is constrained and the rate of encounter should be decreased because of this. In a aquatic habitat it would be harder for the animals to follow the scent trails and the encounter rate is also supposed to be low (Shine 1993). Thus a low encounter rate seems to have been the scenario in the evolutionary history of snakes.

I hypothesize that the polygynous mating system y from the ancestral lizard was not functionally possible due to the difficulty of finding females. A male simply could not easily find more than one female in a season due to the low mobility of the males and the high dispersion of the females. This also offers an explanation for the switch in SSD from male biased to female biased. The ancestor did not have male-male combats or territoriality due to the low encounter rates. While the benefits of large size in females continued (larger clutches, survival, wide range of prey), there was no longer a benefit for males to be large. Furthermore, being small was convenient to move around and find females, and the odds of finding other males was too small; so male combats were no longer a selection force for large size. Due to the low encounter rate between females and males (and perhaps with prey too), females might have not bred very often; thus there was no reason to save energy for the next breeding event. The odds of mortality between one breeding event and the next one were too high, so the females went into larger breeding investments in every reproduction. This would explain the higher RCM found snakes compared with lizards.

6.6 CONCLUSIONS

Studies of snake mating systems are not abundant, but the few that exist have been the product of long term naturalistic field investigations of high quality. Apparently, though, when these articles were written, the genuine interest of the authors in learning about the animals seemed to have surrendered to a more conventional approach to report the findings to fit the accepted style of writing (Greene 1993).

Female anacondas seem to be able to mate multiply in one season while there is no evidence for this in males. Males seem to select mates of high quality making a high investment per mating. A short mating season, high dispersion of the females, and high cost of mobility for the males might account for a “loosely” polyandrous mating system. There is evidence for spermatic competition from the presence of sperm plugs and multiple mating in females. Physical competition in the breeding balls could also be at work. The complicated dynamic of the breeding balls seem to be involved in the remarkable SSD found in this species.

I have shed some light on the anaconda’s mating system but it is still far from being well understood. So far I am unable to determine exactly how males compete for the female, or whether larger males have an advantage in siring more neonates. More field studies involving radio-tagged animals, as well as laboratory experiments, are required to understand such issues as mating success, mate choice, sexual selection forces, natural selection forces, sexual selection gradients, and multiple paternity. DNA analyses are underway to elucidate these issues. A comparative study examining the relative size of the testes among several species of snakes and lizards alike, would help to understand whether sperm competition is an important aspect in the reproductive ecology of the snakes and its evolutionary ancestor.

The previous bias in the pioneer literature regarding mating systems and reproductive investment has led to a lack of understanding of the effect of variance in the female reproductive success in ectothermic vertebrates where females can grow throughout life; and hence their reproductive potential can be a function of their size. Variance in the female’s reproductive success has not been yet considered in the literature perhaps due to the lack of documentation. Thus the potential for a runaway process and effect of sexual selection in females has not been properly considered and indeed it might be the key to explaining some unanswered questions in the ecology and mating systems of snakes.

Current models to explain the evolution of mating systems failed to predict polyandry as a possible scenario. The limited number of species from which the basic natural history is known limits our understanding of mating systems in snakes and seems to be responsible for the misinterpretations in the models developed. I endorse the call of several authors (Madsen and Shine 1993a, Siegel and Ford 1987; Shine 1993, Weatherhead et al. 1995) that long term field studies of individually marked animals in different taxa and different geographic regions are needed to test and develop theories regarding the mating system and sexual selection in snakes. The need to widen our horizons and acquire knowledge of natural history of more species must be retaken in order to increase the predictive power of the models and accomplish a better, more objective understanding of nature.

Table 6-1 Advantages and disadvantages of large size for males and females (Rivas and Burghardt in prep)

	Benefits of large size	Disadvantage of large size
Both sexes	<p>1.- Increased number of prey and the ability to subdue them.</p> <p>2.- Deterrence of predators and reduced vulnerability to them.</p> <p>3.- Lower relative energetic cost and higher thermal inertia.</p>	<p>1.- More easily detected by predators</p> <p>2.- Higher basic maintenance.</p> <p>3.- Lower survival in times of shortage.</p> <p>4.- Higher cost of mobility.</p> <p>5.- Longer maturation time.</p>
Females only	<p>4.- Increased fecundity due to increased coelomic capacity that allows larger clutches and larger breeding investment.</p> <p>5.- Females will have larger offspring with larger chances of survival and faster growth.</p>	
Males only	<p>6.- Increased number of matings and fitness if there is physical competition for the females.</p> <p>7.- Large testis size that by allometric correlation would produce larger amounts of sperm for sperm competition.</p>	<p>6.- Increased cost for moving and dispersing in males that are tracking females for mating.</p>

Table 6-2 Measures of adult anacondas found involved in breeding aggregations. 177 males and 48 females.

	Total Length (cm)				Snout-Vent Length (cm)				Mass (kg)			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Females	370.4	70.6	242.7	517.3	326.2	65.9	210.7	477	32.60	18.59	9.25	82.500
Males	263.2	28.3	188.3	333.7	225.9	24.7	159.3	293.7	6.96	2.07	2.45	14.300



Figure 6-1. Breeding female (Benita, 410 cm TL; 44 kg) at the shore of a canal being courted by 12 males. Photo Tony Rattin.

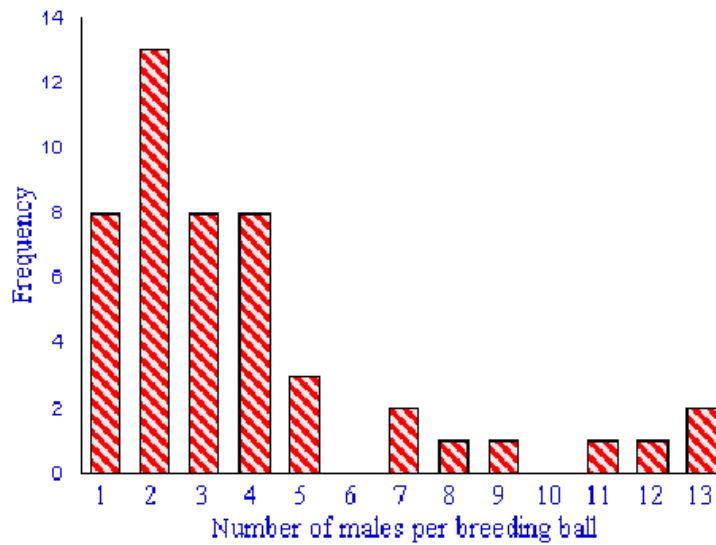


Figure 6-2. Composition (number of males) of the breeding aggregations of anacondas found



Figure 6-3. Mating aggregation of anacondas involving a very large female and some large males. The female moved out of the water and dragged with her some of the males that were coiled around her (A). Other males were removed from their positions and tried to find the female again to continue courtship. However, some smaller males have mistakenly coiled around a very large male and are courting him (B).

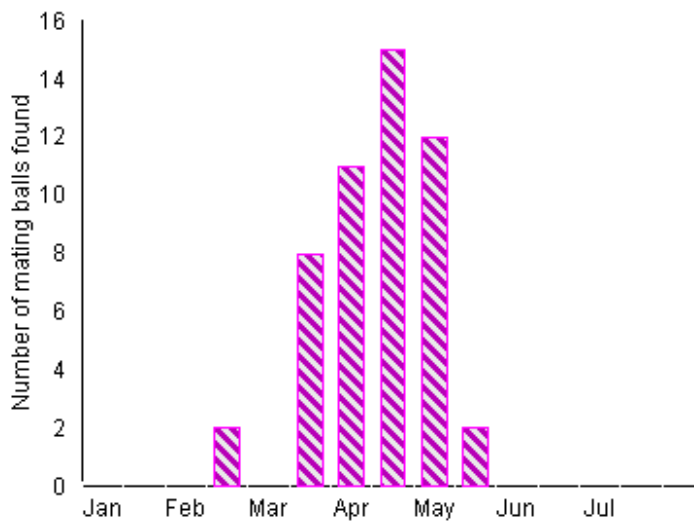


Figure 6-4. Number of breeding aggregations of anacondas found during the breeding season in a locality of the Venezuelan llanos. Each bar represents a two-week period.

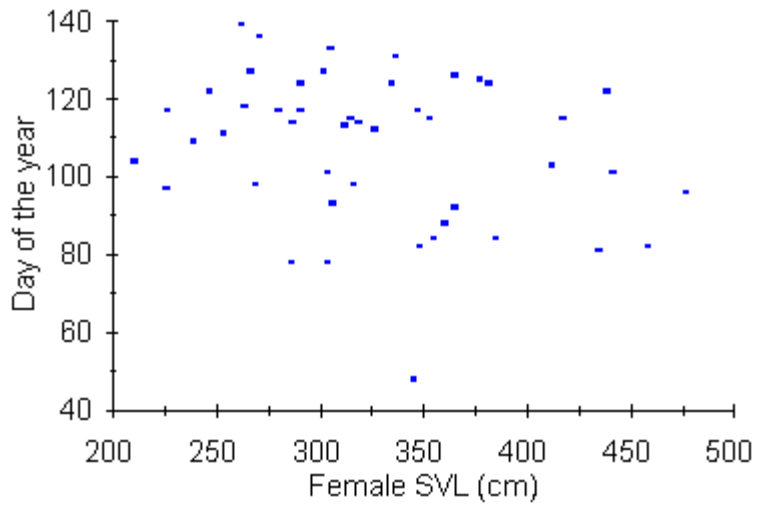


Figure 6-5. Relation between the date in which female anacondas mated and their size. ($r = -0.25$; $p = 0.095$; $n = 46$)

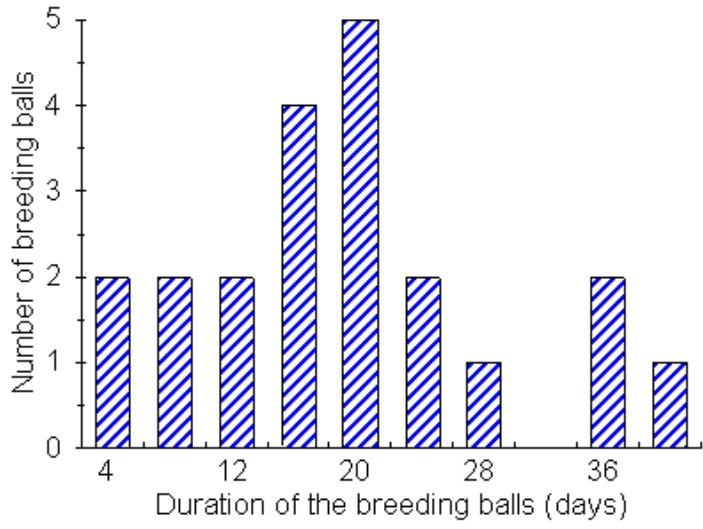


Figure 6-6. Minimum number of days that each breeding ball of anacondas was seen together.

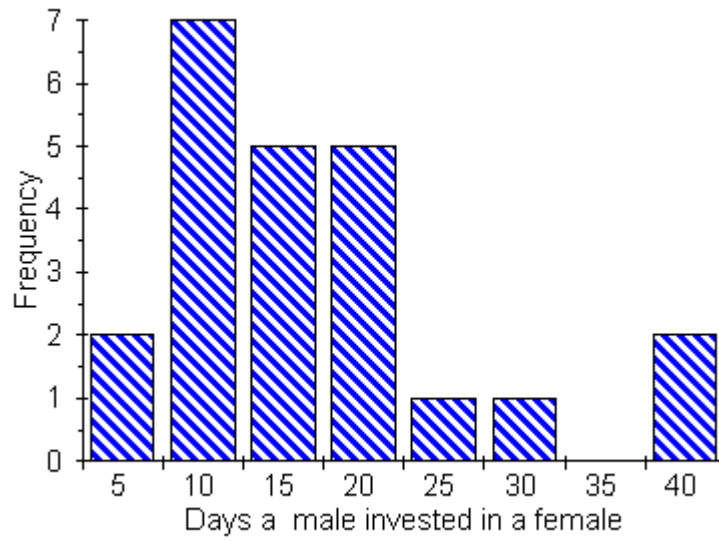


Figure 6-7. Time spent by individual males with a particular female. The time spent was determined by following radio implanted animals or by re-catching them at different times.

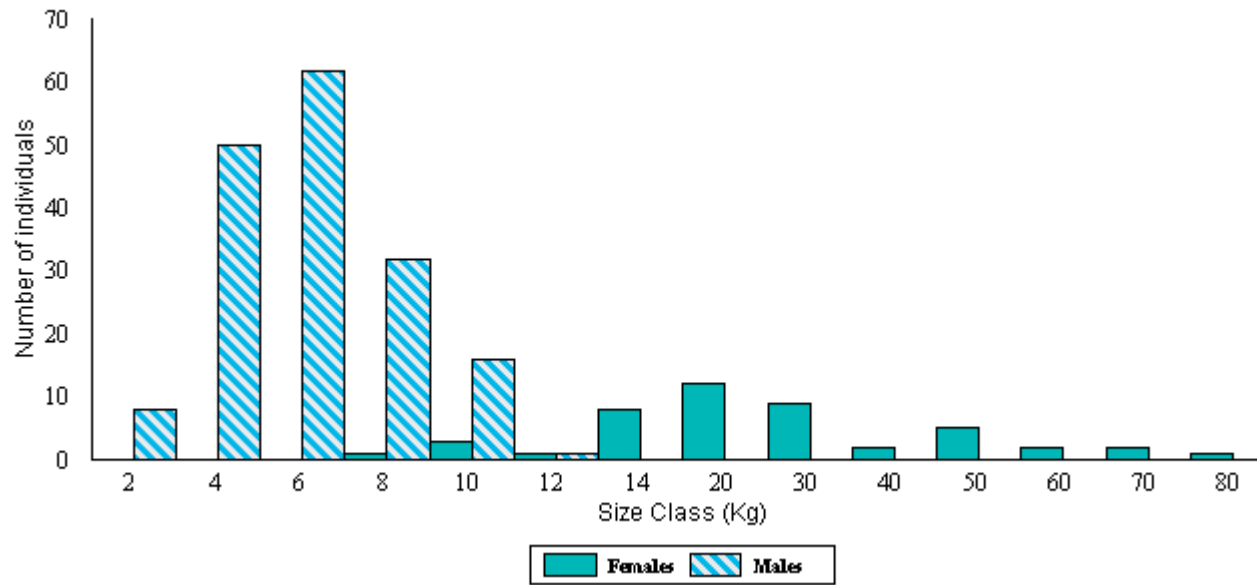


Figure 6-8. Size distribution of the adult population of anacondas. The criteria to determine adulthood was finding them involved in a breeding aggregation. Notice the change in the scale of the “x” axis after 14 Kg.

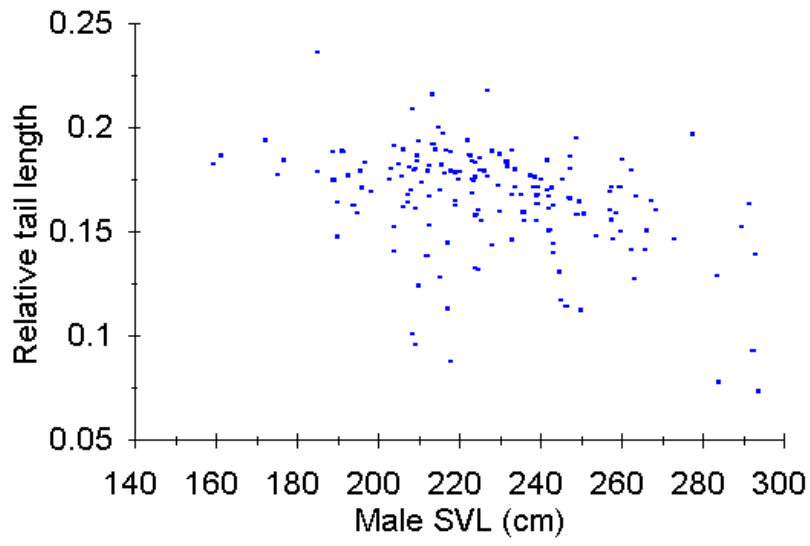


Figure 6-9. Relation between relative tail length (tail length/SVL) of adult male anacondas and the size of the animals. Coefficient of correlation $r = -0.388$, $p < 0.000$; $n = 177$.

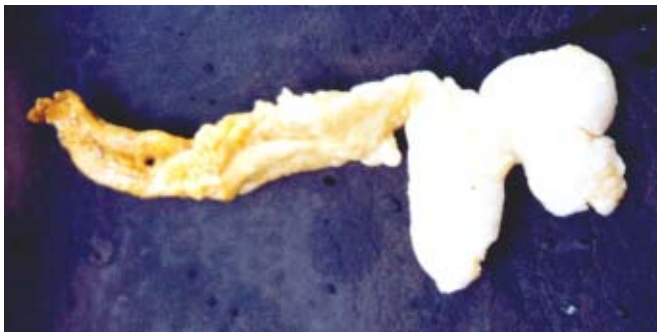


Figure 6-10 Sperm plug obtained from a breeding female anaconda (see text for dimensions).

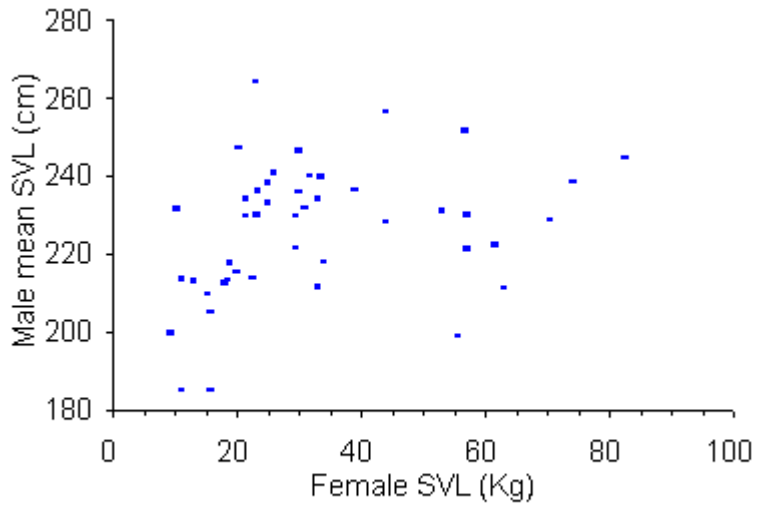


Figure 6-11. Relation of length of the female anaconda and the average length of the males in the breeding ball ($r= 0.38$; $p = 0.009$; $n = 45$).

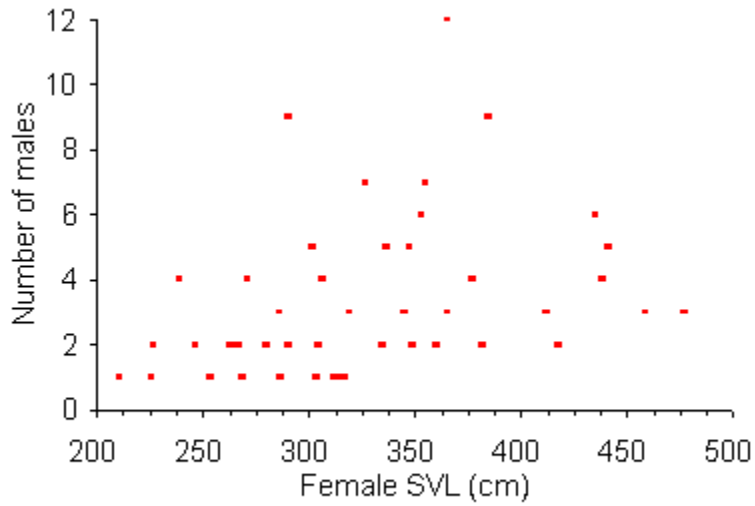


Figure 6-12 Relationship between number of male anacondas in a breeding ball and the length of the female in it ($r= 0.48$; $p= 0.001$; $n= 46$).

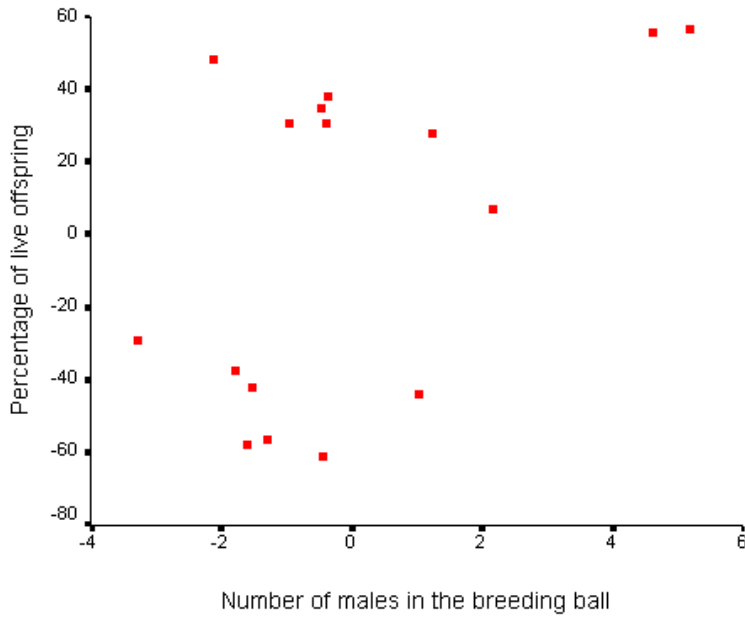
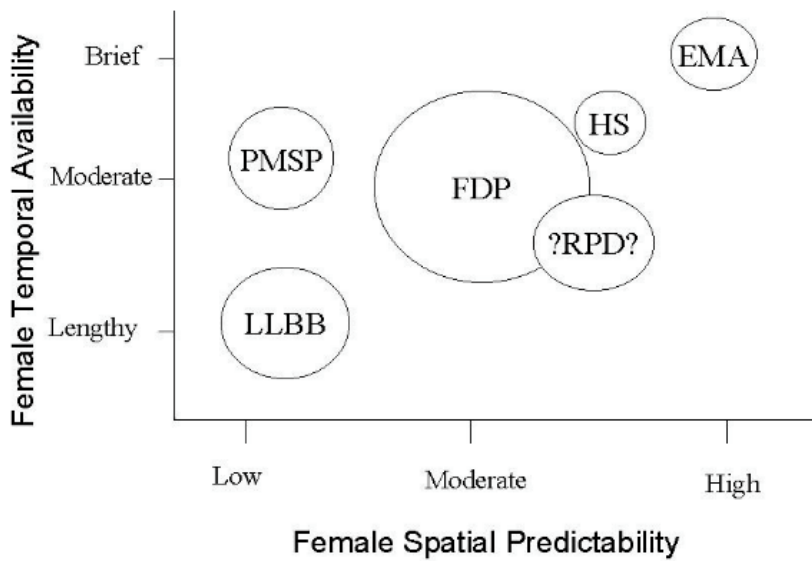


Figure 6-13. Partial regression plot between the proportion of live offspring in female anacondas and the number of males that mated with her holding the effect of female size constant ($r = 0.51$; $0 = 0.027$; $n = 13$, one-tailed test).

Figure 6-14. Mating system predicted by the female temporal availability and the female spatial



predictability by Duval et al. (1993). Female Defense Polygyny (FDP), Prolonged mate search polygyny (PMSP), Explosive mating assemblage (EMA), and hotspot polygyny (HS). They also speculate about the possibility of resource defense polygyny (RDP) as it is present in other taxa. Long-Lasting Breeding Balls (LLBB) appears as a new mating system reported in this contribution.