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Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*

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We report the first field and genetic studies of the reproductive strategies of the Amazonian dart-poison frog *Dendrobates ventrimaculatus*, a species with biparental care. Neither males nor females are strictly monogamous. Males are aggressively territorial, but some females interact without aggression. Monitoring of breeding pools revealed high rates of multiple clutch deposition and high levels of larval cannibalism. Laboratory experiments confirmed larval cannibalism and suggested a benefit to cannibals in increased growth rate. Genetic analyses indicate that offspring from different clutches in or above the same pool vary in relatedness and are on average intermediate in relatedness between individuals from the same clutch and unrelated individuals (from different pools). These data suggest that reproductive parasitism may be common in this species. *Key words:* Amazonian dart-poison frog, reproductive parasitism, reproductive strategies. *[Behav Ecol 8:260–267 (1997)]*

It is now widely recognized that breeding systems evolve as a compromise between the interests of the two sexes, both of which strive to maximize their genetic representation in subsequent generations for minimal costs (Maynard Smith, 1977; Trivers, 1972). Key parameters include direct investment costs, such as yolk production, gestation and lactation, and the costs associated with finding, competing for, and attracting mates.

After birth or hatching, adult behavior may be further modified by opportunities to increase progeny survival through parental care. Parental investment, in the form of care or nutrients, is a valuable resource that may be exploited for genetic gain by an individual's mate, offspring or by individuals outside of the mating pair (Brockmann, 1993; Davies, 1989). Here we use the general term "reproductive parasitism" to refer to cases where one individual exploits the reproductive effort of another for its own genetic gain, at a reproductive cost to the other individual.

Evidence for various forms of reproductive parasitism has been accumulating rapidly with recent developments in genetic analysis (Jeffreys, et al. 1985; Queller et al., 1993). Exploitation can take various forms, including care for unrelated juveniles, usurpation of resources provided for juveniles, and destruction and consumption of juveniles by adults or other juveniles. Evidence of reproductive parasitism has been found in many different taxa, including various species of insects (Field, 1992; van Alphen and Visser, 1990), birds (Birkhead, et al., 1990; McRae and Burke, 1996), and fish (Taborsky, 1994). Reproductive parasitism may be a consequence of limitations on critical resources, without any specifically parasitic strategies (Stevens, 1992), or it may reflect complex adaptive strategies designed to maximize the probability of successful parasitism (e.g., Brown, 1984).

Species with substantial parental care offer particularly valuable opportunities for reproductive parasitism (Brockmann, 1993). Dart-poison frogs are known for their elaborate parental behavior, with maternal, paternal, and biparental care operating in closely related species (Wells, 1981; Weygoldt, 1987). Dart-poison frogs generally produce few offspring, which mature on limited resources in small pools of water such as those that accumulate in leaf axils and treeholes (Myers and Daly, 1983). Various types of care have been observed and include moistening embryos with water (Wells, 1978; Weygoldt, 1987), transportation of tadpoles to pools (Wells, 1981; Weygoldt, 1980), and feeding of tadpoles with infertile eggs (Weygoldt, 1980; Zimmermann and Zimmermann, 1982).

The Amazonian dart-poison frog was originally named *Dendrobates quinquevittatus* (Silverstone, 1975), but populations of this frog from Amazonian Ecuador were re-named *Dendrobates ventrimaculatus* (Caldwell and Myers, 1990). *Dendrobates ventrimaculatus* and its close relative *D. reticulatus* have been studied in terraria, where both species exhibited biparental care (Zimmermann and Zimmermann, 1984; Weygoldt, 1987). Females oviposited above small pools in the leaf axils of bromeliads. Both sexes remained near the eggs during the morning and late afternoon for several days and the female would occasionally shed water on the eggs, moistening them. Once the eggs matured into tadpoles, these were either allowed to slide into the pool above which they were laid or were carried by the male to other pools. After depositing the tadpole in a pool, the male returned to this new pool periodically and called, attracting the female. She then laid eggs, which the tadpoles would eat, in the pool. Older tadpoles also ate younger siblings that happened to slip into the same pool (Zimmermann and Zimmermann, 1984). The same study also showed that tadpoles could survive on other types of food, such as detritus and algae, and hence were not obligate cannibals (Zimmermann and Zimmermann, 1984).

From these observations, it seems that the two species, *D. ventrimaculatus* and *D. reticulatus*, both show biparental care. Davies (1989) pointed out that, although biparental care requires cooperation, it also creates opportunities for exploitation and conflicts of interest. Weygoldt (1987) suggested that male Amazonian dart-poison frogs may attempt to feed the offspring of some of their mates with eggs from subsequent

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mates and that they would accomplish this by scraping eggs from subsequent mates into pools with tadpoles from previous mates; the eggs would be consumed by their half-siblings. This strategy profits the male if the benefits of cannibalism to his older offspring exceeds the cost of losing some younger offspring. It is also clear that benefits would accrue to an individual dart-poison frog placing its own tadpoles in pools containing eggs or younger tadpoles produced by non-relatives. Whether these types of reproductive parasitism occur in the wild is unclear. Here we present results from a field study of mating and parental behavior, along with genetic analyses aimed at determining the extent to which pools contain the progeny of one, or of more than one, pair of frogs.

METHODS

The field sites and field observations

We studied *D. ventrimaculatus* in the rain forest between Pompeya and Limoncocha, near the Napo River in eastern Ecuador. We established two field sites in areas of high frog density and marked a transect along a trail through each site every 2 m, for a total length of 100 m. The study sites extended approximately 20 m to either side of the trail. We began making observations on 16 June 1993 and continued through 12 August 1993. Unfortunately, one of these study sites was destroyed by flooding halfway through the research.

We captured and marked (by toe-clipping) adult frogs found in the study sites. We measured the snout-vent length of each frog using dial calipers and weighed each frog using a portable electronic scale (Ohaus model CT200). Limited sexual dimorphism exists, females typically being larger and more rotund than males. However, there is some overlap, and size was not a reliable way to sex the animals. Hence, we only recorded the sex of adults if they were caught while courting or for males if they were found calling. Almost all individuals that were collected were sexed by dissection (two individuals had to be preserved rapidly, and hence there was no time for dissection).

We made observations by quietly patrolling the study area and recording the identity, behavior, associations and interactions of all frogs seen. These frogs are diurnal, and we carried out observations between 0800 and 1800 h. At the study site, frogs deposited eggs above small pools of water that formed in the stem axils of *Heliconia* plants. These eggs would gradually mature in the axil. In some cases, the tadpoles would hatch in the jelly mass above the pool and either gradually slide into the pool below or be carried by an adult to another pool. Clutches of embryos were often submerged in the pool before hatching because of changing water levels and because of sliding. In the rest of the paper, we will refer to clutches (and embryos) as being "in axils" whether they were currently above or submerged in the pool within the axil. We censused these axils by examining the inside of the axil with a small halogen flashlight. The frogs also carried tadpoles from a clutch above one pool and deposit them into another pool. All axils in the study sites that contained embryos or tadpoles were marked with plastic flags and checked daily, both for new depositions and to follow the fate of previously deposited clutches. Different clutches within each *Heliconia* axil could be distinguished because each clutch is contained within its own, distinct, jelly mass. We also were able to collect a single embryo from each clutch when it was first discovered, and then follow the fate of the rest of the clutch. We removed small insects (e.g., beetles and earwigs) found in some of the axils to make inspection easier.

At the end of the two-month study, we recorded the number of surviving tadpoles in each axil. For genetic analysis, the following samples were collected: one embryo from every newly deposited clutch; at the end of the study, all tadpoles from all axils that were observed to have more than one deposition; all adults found (during the last week of the study) in or near axils with more than one deposition; all larval stages from axils found off the study sites that contained individuals from more than one clutch. The study and collections were carried out with the permission of the Ecuadorian Ministry of Natural Resources, and a permit for exportation of specimens was obtained from that ministry (CITES permit number 024 IC).

Cannibalism

We investigated the importance of cannibalism through a series of experiments. We placed pairs of tadpoles, or a tadpole and an embryo, together in individual plastic cups containing a quantity of water similar to that contained in the axils normally used by the frogs (approximately 50 ml). We placed small amounts of leaves and detritus in the cups, but not so much that it was difficult to observe the tadpoles or embryos. Each pair consisted either of a tadpole and an embryo or two unequal sized tadpoles. The two participants came either from the same ($n = 15$) or from different pools ($n = 12$). Replicate numbers were limited by the availability of larval stages. During one week we inspected the cups daily for evidence of cannibalism.

Microsatellite cloning

We developed microsatellite markers to measure relatedness between individuals or categories of individuals. We obtained microsatellite markers essentially following the methods of Rassmann et al. (1991). We ligated size-selected fragments of dart-poison frog DNA into a Bluescript KS vector and then transformed them into E. coli, strain TG1. Recombinant colonies were screened using a synthetic dinucleotide AC probe. We miniprepped and sequenced (either manually or with an ABI Automated Sequencer) positively hybridizing colonies. We then designed DNA primers against unique DNA sequences flanking promising microsatellite regions.

Statistical methods

Estimation of allele frequencies and the frequency of null alleles
Only two of the many loci cloned proved suitable for sample screening. In addition, both loci we used carry alleles that fail to yield an amplification product (non-amplifying or null alleles). Individuals carrying one null allele appear as phenotypic homozygotes, whereas individuals that are homozygous null fail to yield any product at all and are generally scored as intransigent samples.

We used a likelihood approach to analyze our data. By so doing, a probabilistic allowance can be made for the presence of null alleles. We estimated allele frequencies using the following method, with the implicit assumption that genotype frequencies are in approximate Hardy-Weinberg equilibrium: For any given allele, a, the number of individuals that carry at least one copy, na, relates to the true frequency of that allele as follows:

$$na = Nf_a^2 + 2Nf_a(1 - f_a) \quad (1)$$

where f_a is the frequency of allele a and N is the total sample size.

Since n_a is known, f_a can be derived by solving the quadratic equation. At a locus where there are null alleles, the number of phenotypic a homozygotes (those showing a single a allele) will on average include some true aa homozygotes and some

individuals that are heterozygous for a null allele, here denoted *ao*. However, the expected number of *aa* homozygotes is given by Nf_o^2. Consequently, subtracting Nf_o^2 from the observed number of phenotypic homozygotes will yield an estimate for the number of *ao* heterozygotes. By repeating this process for each allele in turn, we arrive at an estimate for the total number of null/normal heterozygotes, n_{no}. Conversely, the number of individuals that do not carry a null allele, n_{nn}, is given by:

$$n_{nn} = N - n_{no} \quad (2)$$

Both n_{no} and n_{nn} can be expressed in terms of the frequency of null alleles, f_o:

$$n_{no} = 2Nf_o(1 - f_o) \quad (3)$$

$$n_{nn} = N(1 - f_o)^2 \quad (4)$$

where f_o is the frequency of the null allele.
Dividing Equation 3 by Equation 4 and canceling we obtain

$$\frac{n_{no}}{n_{nn}} = \frac{2f_o}{1 - f_o} \quad (5)$$

and, by rearrangement,

$$f_o = \frac{n_{no}}{(2n_{nn} + n_{no})} \quad (6)$$

Since we have estimates of both n_{no} and n_{nn}, using Equation 6 we can estimate f_o.

Wherever null alleles occur, there is also the possibility of null homozygotes (*oo*), individuals whose DNA will fail to yield any product. Such individuals will cause us to underestimate the sample size. Simply counting all DNA samples that fail to amplify as null homozygotes does not work because some DNA samples are naturally intransigent for any of a variety of reasons. However, the true sample size, N', can be estimated as follows:

$$N' = \frac{N}{(1 - f_o^2)} \quad (7)$$

The value of N' then replaces N and the whole process iterated, each time improving the estimates of the frequencies of all alleles, both visible and null.

Measurement of relatedness

In any given comparison between two individuals, non-relatives will share fewer alleles than relatives, and the alleles non-relatives share will tend to be only those that are common. Conversely, relatives will tend to share alleles more often than expected by chance alone. Consider a locus at which the paternal alleles of two young animals have been identified. How likely are these animals to share the same father? There are two possibilities: either the paternal alleles are the same or they differ. The probability of observing two different alleles in non-relatives, P_{nr}, is given by $2f_if_j$, where f_i and f_j are the frequencies of the two alleles, i and j. The probability of these same alleles being inherited through shared paternity, P_r, is given by the product of the probability of observing an ij father ($=2f_if_j$) and the probability of the paternal alleles segregating such that the two offspring carry different alleles ($= .5$). The ratio of the probabilities of these two alternative scenarios is thus

$$\frac{P_{nr}}{P_r} = \frac{2f_if_j}{f_if_j} = 2 \quad (8)$$

In other words, regardless of the frequencies of the alleles concerned, it is twice as likely that two different alleles come from different, rather than the same, parent.

Similar calculations can be made for the situation when two alleles are the same. For unrelated alleles, P_{nr} is given by f_i^2. For shared paternity the calculation is a little more complex since there are two possible scenarios: the father can be genotype *ii* or *ix*, with respective probabilities f_i^2 and $2f_i(1 - f_i)$, where x is any allele other than the one observed. With respect to segregation, all offspring born to *ii* fathers will share the i allele, but only a quarter of all pairs of offspring born to fathers who are genotype *ix* will share the i allele. Thus,

$$P_r = f_i^2 + 0.5f_i(1 - f_i) \quad (9)$$

and, for alleles which are the same,

$$\frac{P_{nr}}{P_r} = \frac{f_i^2}{f_i^2 + 0.5f_i(1 - f_i)} = \frac{2f_i}{(1 + f_i)} \quad (10)$$

Equation 10 behaves as expected: the rarer the allele that is shared, the smaller becomes P_{nr}/P_r and the less likely it is for the two alleles to derive from different fathers.

At any given locus, each individual carries two alleles, one inherited from its mother and one inherited from its father. In a comparison between two individuals, therefore, four possible allelic comparisons can be made: one between paternal alleles, one between maternal alleles, and two involving one paternal and one maternal allele. Ideally, to assess shared parentage, an index of relatedness would be based only on the relevant one of the former two categories; for example, the paternal-paternal comparison to assess shared paternity. However, the paternal origin of each allele is unknown. Consequently, our index of relatedness, R, is based on all four possible comparisons, being the log of the product of four P_{nr}/P_r values.

$$R = \log\left\{\prod_4^1 \frac{P_{nr}}{P_r}\right\} \quad (11)$$

In so doing, we are making the assumption that the three irrelevant comparisons that have to be made are contributing only background noise.

Estimating the proportion of each class of relatedness

We are interested in five different sets of comparisons: (a) between progeny from different pools, (b) between progeny from different clutches in the same pool, (c) between progeny from the same clutch, (d) between progeny and an associated female (possible mother), and (e) between progeny and an associated male (possible father). We have typed 193 individuals from 38 pools above which more than one clutch was laid, including 12 full-sibships (individuals from the same clutch).

To interpret our genotype data, we began by using the allele frequency distributions to derive theoretical *R*-value distributions for each of the four primary classes of relatedness: full-sibs (F), parent-offspring (P), half-sibs (H) and unrelated (U). With only two loci it is feasible to use a simple computer program to produce a "true" distribution based on the probabilities of observing all possible segregation patterns for all possible parental combinations. With more loci it would be sufficient to approximate the true distribution using computer simulation, sampling the observed allele frequency distributions to produce say 10,000 progeny in each relatedness class.

Having derived the four primary expected *R*-value distributions, we next calculated hybrid *R*-value distributions for all possible mixtures of relatedness classes at 10% intervals. For example, if an *R*-value of 3 occurs with probabilities .5, .2, and .1 in U, H, and F classes respectively, a group of comparisons containing 60% U, 20% H and 20% F would be expected to

yield an *R*-value of 3 with a probability .35 (= .5 × .6 + .2 × .2 + .2 × .1). In this way, we calculated the probability of observing all possible *R*-values for all possible mixtures of relatives.

To assess the most likely proportions of different classes of relatives represented by a set of *R*-values, we use a likelihood approach. Using each of the hybrid distributions in turn, we calculated the probability of obtaining the empirical *R*-values. The best-fit set of proportions is then the one with the highest probability. Ninety-five percent confidence limits are generally taken as being all values that lie within 2 log units of the best fit. Results are displayed in tabular form, one axis being the proportion of unrelated comparisons and the second axis representing the proportion of half-sibs. The proportion of full-sibs is then the remainder (100% minus the proportion of unrelateds minus the proportion of half-sibs). These methods are described in greater detail elsewhere (Amos W, Cooper J, and Summers K, manuscript in preparation).

Treatment of null alleles and intransigent samples

Where any sample is typed as a phenotypic homozygote, it could either be a true homozygote or a null/normal heterozygote. Similarly, when an individual gives no signal, this may be because the sample failed to amplify, or it may be because that individual is a null/null homozygote. To overcome these ambiguities, we elected to apportion part of each individual based on the probability of each possible scenario. To illustrate, if the probability of a phenotypic homozygote being a true homozygote were to be .75 and that of being a null heterozygote were .25, and the *R*-values for each of these two scenarios were x and y, respectively, then this comparison would be scored as .75 of a comparison scoring x and .25 of a comparison scoring y. In the case of null homozygotes versus intransigent samples, appropriate proportions are calculated and the fraction attributable to intransigence then discarded.

We also estimated average degrees of relatedness using the program Relatedness (Goodnight and Queller, 1994). Although some of the assumptions underlying the algorithms employed in the Relatedness program are violated by the presence of null alleles, we think it is useful to compare our maximum likelihood estimates with estimates obtained with a different method. We used Relatedness to estimate average degrees of relatedness between classes of individuals (e.g., the average relatedness between individuals from the same clutch), rather than between particular individuals. To determine average relatedness between putatively unrelated individuals, all possible comparisons were made between one individual from each of 38 pools, chosen without replacement. This procedure was repeated five times, and the average of the five averages was used.

RESULTS

Site faithfulness and territoriality

During the study period, we captured and marked 167 frogs on the first study site. Of 77 individuals for which sex could be determined, 30 were females and 47 were males. The preponderance of males may have more to do with their ease of detection (i.e., through hearing them call), then with any sex-ratio bias in the population. We recaptured nine females once and three twice. The number of days between recaptures gives a minimum estimate of site fidelity for recaptured individuals. The average number of days between recaptures for females was 17.2 and ranged from 3 to 34 days (SE = 8.9). We recaptured 20 males once and five twice. The average number of days between recaptures of males was 22.3, and ranged between 2 and 49 days (SE = 13.4). Of 87 adults of unknown sex, we recaptured 19 once and one was recaptured twice (Range = 1–45, SE = 13.1). The distance between recaptures of females averaged 5.1 m (SE = 3.9, N = 9). The distance between recaptures of males averaged 2.0 m (SE = 1.7, N = 31). The distance between recaptures of all individuals averaged 2.8 m (SE = 2.7 m, N = 62). These data indicate that at least some individuals are site faithful for more than a month. The difference between the average recapture distance for males and females is marginally significant using a t test (t = 2.52, N = 40, p = .052).

Calling males were aggressive toward each other. Aggression involved calling back and forth and direct physical fighting, in which each male attempted to wrestle his opponent to the ground or knock him off his perch. We observed fights between males three times during the study. Males also were observed to chase other males out of their area, and once we observed a male chase a tadpole-carrying male from a pool that the second male had tried to explore. This may have been an attempt to prevent the tadpole-carrying male from depositing a tadpole in a pool used by the other male. We did not observe any aggression between females.

Courtship and mate fidelity

We carried out focal follows (Altmann, 1974) on courting couples. Because of time constraints imposed by the design of this study, the majority of these focal follows were short (5 min). Afterward, we captured the couple for identification and marking and then released them. We made an exception for one pair that was followed for 75 min. Also, a complete courtship was observed during a pilot study 3 km away near Limoncocha in 1988.

Courtship typically consisted of the male leading the female by hopping a few centimeters away and then calling. The female would then approach the male, whereupon he would turn his back to the female, and she would stroke his legs and/or dorsum with her forefeet. He would then hop forward again, repeating the process. The courtship observed in 1988 lasted for 9 h before the female oviposited. These observations are similar to those reported by Zimmermann and Zimmermann (1984) on captive animals, who reported that courtship could last up to 24 h. They also noted that males "dance around" females.

Recaptures of individuals as members of courting pairs provided information concerning mate fidelity. In total, we captured 64 courting pairs, and mate fidelity appeared to be low. In 10 of 15 cases where we recaptured a male as a member of a courting pair, he was courting a different female, and four times males were observed to court with two females simultaneously. We recaptured 10 females as members of courting pairs, and in half of these cases the female was courting with a different male. These results suggest that monogamy is not the rule in *D. ventrimaculatus*; males and females are apparently willing to mate with more than one partner within a few weeks.

Egg and tadpole deposition

Tadpoles from some clutches were carried away before they slipped into the pool below, but most clutches went into the pool above which they were deposited. A total of 109 axils showed evidence of deposition (embryos or tadpoles) and were monitored for varying amounts of time, depending on the date they were discovered (mean = 24 days, SE = 15 days, range = 3–57 days). Even so, we found embryos or tadpoles of clearly differing ages in 50 axils, indicating that individual axils often receive more than one deposition (in one case, we found five separate depositions in a single axil). We do not

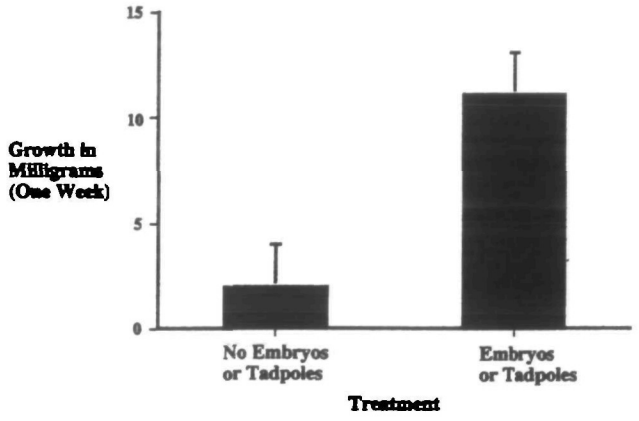

Figure 1
Growth of tadpoles in feeding experiments. Tadpoles were measured and weighed and put in plastic cups for one week. Tadpoles in the embryo treatment were provided with detritus and three eggs, embryos, or smaller tadpoles. Tadpoles in the no-embryo treatment were provided with detritus only. Tadpoles from both treatments were re-measured and re-weighed after one week.

know how many different adults typically place offspring in an axil, but several different adults were caught in many of the axils during the study. Considering only those axils where adults were caught, an average of 2.1 adults were found at each axil ($N = 63$ pools, SE = 1.4). We caught more than two adults in 14 axils, and up to eight were caught in a single axil during the study.

We monitored and collected 40 axils with multiple deposition. An average of 1.2 embryos were deposited per axil per week. This estimate represents a minimum, since cannibalism and predation will reduce the number of observed embryos and tadpoles. At the end of the study, 25 axils had one surviving tadpole, 11 had no tadpoles, and four had two tadpoles. This pattern suggests that cannibalism is common and that starvation, predation or death from other causes play a significant role.

Cannibalism

We observed cannibalism both when the larvae came from different axils and when they came from the same axil, occurring in 10 of 12 different axil replicates and in 7 of 15 same axil replicates. Embryos were more likely to be eaten than tadpoles (15 of 19 embryos, 2 of 8 tadpoles, $G2 = 4.15$, $p < .05$, $N = 25$), suggesting that tadpoles are more difficult to kill and eat. Excluding cases in which two tadpoles were used (which would bias the results), there was a non-significant trend toward tadpoles being more likely to eat embryos from different axils than embryos from a different clutch in their own axil (9 of 9 from a different axil were cannibalized, 6 of 10 from the same axil were cannibalized; Fisher's Exact test, $p = .09$, $N = 19$). However, our small sample size means that the power of this test is poor, and hence we feel it would be premature to exclude the possibility that cannibalism is more likely between individuals from different axils.

We also examined the effect of cannibalism on growth rate by comparing the growth rate of cannibals given at least one tadpole or embryo to eat with that of tadpoles not given tadpoles or embryos. Having begun with no significant difference in mass between the two treatment groups, the cannibals grew at a significantly higher rate during a week (Figure 1, Kruskal-Wallis statistic = 7.95, $p < 0.02$, $N = 14$), suggesting a significant nutritional benefit to the cannibal. The control pools contained detritus, but not other nutrients that could contribute to tadpole growth in the field (e.g., mosquito larvae). Hence, our study suggests that cannibalism increases growth rate in the field, but is not conclusive.

Microsatellite analysis

The results of the maximum likelihood analyses are presented in Tables 1–3. Table 1 shows the log likelihood values calculated for within-clutch comparisons. Best fit is found with approximately the following proportions: 20% unrelateds, 20% half-sibs, and 60% full-sibs, with the highest values lying in the top half of the table. The analysis suggests that the levels of band sharing are not as high as would be expected if all individuals from the same clutch were related as full-sibs, but the sample size was quite small, and the 95% confidence limits (2 log units either side of the best fit) include 90% full-sibs and 10% half-sibs. Members of a single clutch could be half-sibs because of either multiple fertilization or because more than one female placed eggs in a single clutch. Given that males are extremely aggressive, and a single male was observed courting two females simultaneously four times, the latter possibility seems more likely. Table 2 shows likelihood values for comparisons between clutches within pools. Best fit

Table 1
Maximum likelihood analysis of the proportions of different classes of relatives within clutches

Unrelated	Half-sibs										
	0	1	2	3	4	5	6	7	8	9	10
0	−3.0	−1.5	−0.6	−0.1	−0.0	−0.2	−0.7	−1.5	−2.7	−4.4	−7.0
1	−1.3	−0.5	−0.1	0.0	−0.2	−0.7	−1.6	−2.8	−4.5	−7.1	
2	−0.4	−0.0	0.0	−0.2	−0.7	−1.6	−2.8	−4.6	−7.2		
3	0.0	0.0	−0.2	−0.8	−1.6	−2.9	−4.7	−7.4			
4	0.0	−0.2	−0.8	−1.7	−3.0	−4.8	−7.5				
5	−0.3	−0.9	−1.8	−3.1	−4.9	−7.7					
6	−0.9	−1.8	−3.2	−5.0	−7.9		Rest =				
7	−1.9	−3.3	−5.2	−8.1			Full-sibs				
8	−3.4	−5.3	−8.3								
9	−5.5	−8.5									
10	−8.8										

Log likelihoods are shown for the number of half-sibs, full-sibs, and unrelated individuals out of 10. Values less than two log units below the maximum value are within the 95% confidence limits. $N = 27.15$. The sample size is a fraction because it reflects the most probable value, allowing for mistypings (recalcitrant samples).

Table 2
Maximum likelihood analysis of the proportions of different classes of relatives between different clutches in the same pool

Unrelated	Half-sibs										
	0	1	2	3	4	5	6	7	8	9	10
0	−54.1	−38.9	−20.0	−19.7	−13.4	−8.5	−4.9	−2.4	−1.2	−1.4	−3.3
1	−37.5	−26.9	−18.8	−12.6	−7.8	−4.3	−2.0	−0.9	−1.1	−3.2	
2	−25.9	−18.0	−11.9	−7.3	−3.9	−1.6	−0.6	−0.9	−3.1		
3	−17.4	−11.4	−6.8	−3.5	−1.3	−0.4	−0.7	−3.0			
4	−10.9	−6.5	−3.2	−1.1	−0.2	−0.6	−3.0				
5	−6.1	−3.0	−0.9	−0.1	−0.6	−3.1					
6	−2.8	−0.8	−0.0	−0.6	−3.1						
7	−0.7	0.0	−0.6	−3.3			Rest =				
8	−0.0	−0.7	−3.5				Full-sibs				
9	−0.8	−3.7									
10	−3.9										

Log likelihoods are shown for the number of half-sibs, full-sibs, and unrelated individuals out of 10. Values less than two log units below the maximum value are within the 95% confidence limits. $N = 128.7$.

is achieved with approximately 70% unrelated, 10% half-sibs, and 20% full-sibs, with the highest values lying in the bottom half of the table. The 95% confidence limits include neither 100% nor 0% unrelateds. This suggests that the study pools contained both related and unrelated clutches. However, the poor discrimination between full- and half-siblings means that we cannot say whether relatedness between broods reflects multiple depositions from a single male-female pair or faithfulness to one pool by a single male (or possibly female) who has mated with more than one other individual. Table 3 shows log likelihood values for relatedness between males and females and larvae from the axils near which they were caught. Best fit suggests that there is little difference between the sexes. Both males and females spend time near axils in which an average of 40%–50% of larvae are their own. Considered in isolation, these values are compatible with a spectrum of scenarios, from all clutches containing 40% of each attending frog's progeny through to frogs spending 40% of their time near axils that contain only their progeny. However, given the levels of relatedness we have found for between-clutch comparisons within the same pool, the most likely situation appears to lie nearer to the mixed-clutch scenario than to the pure-clutch alternative.

The results of the analysis of average degrees of relatedness between classes of individuals using the Relatedness program are shown in Figure 2. This figure shows a comparison between average degrees of relatedness among putative full-siblings (from the same clutch), among individuals from different clutches in the same pool, and among putative unrelated individuals (individuals from or associated with different pools). Individuals from different clutches in the same pool were on average intermediate in relatedness. There was a significant difference between the estimate of relatedness between full-siblings and the estimate for unrelated individuals [full-siblings = .57 (SE = 0.17), unrelated = .02 (SE = 0.05), Mann-Whitney $U = 165.5$, $p < .05$, Nsibs = 12, Nunrelated = 5]. However, the distribution of relatedness estimates between individuals from different clutches in the same pool was not significantly different from that of full-siblings [within pool, between clutches = .22 (SE = 0.08), Mann-Whitney $U = 149.5$, $p = .09$, Npool = 38], nor from that of unrelated individuals (Mann-Whitney $U = 114.5$, $p = .39$). This is because the estimates for relatedness within pools and between clutches varied widely, from quite low to very high, with some skew

Table 3
Maximum likelihood analysis of the relatedness between males and females associated with particular pools and the eggs, embryos, and tadpoles found in the pool

	Male	Female
0	−1.83	−1.81
1	−1.01	−0.97
2	−0.48	−0.45
3	−0.16	−0.14
4	−0.01	0.00
5	0.00	−0.01
6	−0.12	−0.14
7	−0.38	−0.41
8	−0.76	−0.81
9	−1.34	−1.34
10	−2.12	−1.95

Log likelihoods are shown for the number of relatives (presumed offspring) and non-relatives out of 10. Log likelihoods two or more units below the maximum value are below the 95% confidence limits. $N = 42.19$ for females and 35.19 for males.

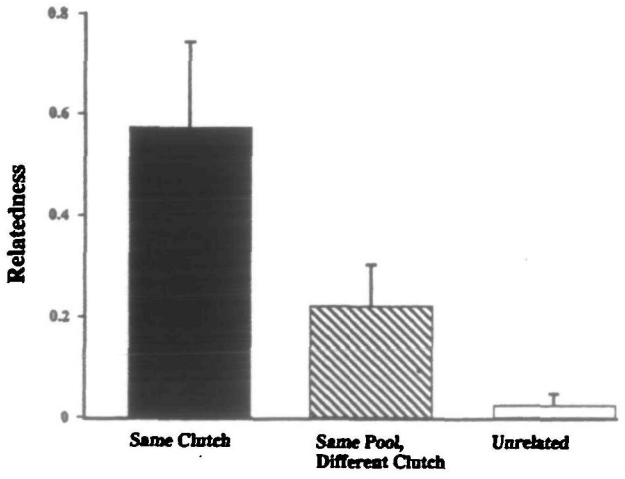

Figure 2
Average level of relatedness between members of the same clutch (black bar), members of the same pool but different clutches (striped bar), and members of different pools (white bar). Relatedness was calculated by the program Relatedness (Queller and Goodnight, 1994). Standard errors were determined using a jackknifing procedure implemented in the program Relatedness.

toward higher relatedness. This suggests a mixture of related and unrelated individuals from within the pools, a result that is consistent with the estimates of relatedness between clutches within pools that we obtained with the maximum likelihood method.

DISCUSSION

Our evidence suggests that reproductive parasitism and cannibalism play an important role in the breeding system of Amazonian dart-poison frogs. Our study is incomplete, and sample sizes are small: lab difficulties, time constraints, natural disasters (e.g., the loss of one study site because of flooding), and the difficulty of working in a remote region of the tropics all contributed. Nevertheless, we think that our data suggest heretofore unappreciated complexities in the reproductive strategies of these frogs.

Observations of adult behavior suggest that some males are site faithful and remain in the same area for well more than a month. Females, as well, were recaptured in the same area up to a month after their first capture, suggesting that at least some are site-faithful. The data on distance between capture sites suggest that females may have larger home ranges than males, but this needs further investigation.

Multiple deposition is common; many axils have embryos or tadpoles from more than one clutch simultaneously. At the same time, tadpoles or embryos from different clutches in the same axil are frequently unrelated or related as half-siblings. This indicates that more than two genetically unrelated individuals frequently have offspring in the same axil. Given an opportunity, tadpoles eat younger tadpoles or embryos. Larval cannibalism occurs in other species of dart-poison frogs, as well as other species of anuran amphibians (Crump, 1992). In the Amazonian dart-poison frog, cannibalism appears to be the major cause of mortality within the pools. It is unlikely that the high rates of mortality were caused by predation by other species since the most likely predators would be organisms such as damselfly larvae, but these were not found in any of the pools and tend to occur only in larger pools (Fincke, 1992). Also, it seems unlikely that predators would consistently eat all but one or, occasionally, two tadpoles.

Cannibalism may also contribute significantly to tadpole growth rate, as indicated by our experiments in plastic cups. *Dendrobates pumilio*, a related species of dart-poison frog from Panama, has female parental care in which females provide nutritive eggs to their offspring (Weygoldt, 1980). *Dendrobates pumilio* tadpoles will not grow or survive without receiving trophic eggs from their mothers (Brust, 1993). The question now arises as to how dependent *D. ventrimaculatus* tadpoles are on cannibalism. One indication may be given by pool size: *D. pumilio* use pools that are similar in size to those used by *D. ventrimaculatus* at Limoncocha. It therefore seems possible that *D. ventrimaculatus* tadpoles find it difficult to complete metamorphosis without eating some other tadpoles or embryos. On the other hand, in another species of dart-poison frog *D. auratus*, which shows male parental care and also deposits more than one tadpole in the same pool, tadpoles can grow and survive without receiving other tadpoles to eat (Summers, 1990). In this case the pools tend to be larger (e.g., treeholes).

If, as seems likely, cannibalism and reproductive parasitism play an important role in *D. ventrimaculatus* breeding behavior, a number of predictions can be made. First, it must be advantageous, if at all possible, to distribute progeny so as to maximize the probability that they cannibalize rather than end up being cannibalized. On the one hand, this implies movement so as to locate suitable pools containing younger embryos. On the other hand, an advantage will be gained by any frogs that manage to prevent depositions of older tadpoles in pools where they themselves have progeny. In other words we should expect to find evidence of male mobility and aggression between males. Both these predictions appear to be supported. Males are territorial and aggressive toward other males that attempt to call in the same area. Much of this aggression may be associated with mating effort, as in some species with male parental care (e.g., Summers, 1989, 1992). However, the observation of a male chasing another, tadpole-carrying male from a pool suggests that males also specifically attempt to keep other males from depositing in pools within or near their territories.

By contrast, the behavior of females remains unclear. Zimmermann and Zimmermann's (1984) report that female *D. ventrimaculatus* in captivity are aggressive toward other females, and previous research on other species with male parental care indicates female aggression is common (Summers, 1989, 1992). However, we never observed aggression between females, even though two females were seen courting with a single male four times, suggesting that this lack of aggression cannot simply be ascribed to a lack of opportunity.

The observation that individuals of both sexes often court, and presumably mate with, more than one partner suggest that monogamy is not the rule in this species. This is consistent with Weygoldt's (1987) hypothesis that males will attempt to be polygynous. Females, as well, may profit from polyandry.

Experiments are planned to investigate whether individuals avoid placing embryos in axils that already contain tadpoles and whether they attempt to place tadpoles in pools with embryos or smaller tadpoles. These experiments should reveal whether reproductive parasitism in these frogs is the result of conflicting adaptive strategies actively pursued by individual adults or an epiphenomenon of constraints on pool availability, in which specific parasitic strategies play little or no role.

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