Many behavioral traits have a relatively wide range of expression. In fact, these behavioral traits typically are more variable than are related morphological characteristics. A snake may freeze or flee in response to a predator, for example, but the number of that snake’s ventral scales does not change (Brodie and Russell, 1999). Variability of behavioral expression presumably permits a more flexible response to changing environmental or social cues. This flexibility is documented by studies of behavioral thermoregulation in lizards: in a great many species, individuals have a remarkable behavioral ability to maintain body temperature precisely at the preferred level, despite extreme thermal patchiness in the environment (Huey and Pianka, 1977).

In terms of social cues, behavioral flexibility may be particularly important at the level of male–female reproductive interactions. Potential female mates, for example, may vary in their levels of receptivity. A male that adjusts his behavior to provide the level and type of stimulation required by a particular female presumably would be more likely to sire offspring (see Boake, 1989). A prime example of such an adjustment has been observed in Satin Bowerbirds (Ptilonorhynchus violaceus) in which a male may encounter females that differ in their sensitivities: young females mating for the first time often are intimidated if a male’s display is too intense, whereas experienced females are more likely to respond favorably to a high intensity display. After a brief assessment, the male bowerbird quickly adjusts the intensity of his displays (Patricelli et al., 2004; Coleman et al., 2004).

In amphibians, the potential for flexibility and consistency in male courtship behavior has been studied in detail in newts (Hilliday, 1975), as well as in several species of lungless (plethodontid) salamanders (Arnold, 1977; Houck and Verrell, 1993; Verrell and Mabry, 2000). These salamanders show consistency in that most courtships show a highly predictable pattern of female courtship interactions (Houck and Arnold, 2003). In plethodontids, for example, courtship behavior for all known species is characterized by a “tail-straddling walk:” the female places her head on the dorsal surface of the male’s tail and her fore- and hind limbs straddle his tail (see fig. 1 in Arnold, 1976). In this position, the male and female walk forward in tandem, ultimately leading to spermatophore deposition and sperm transfer. Another consistent trait in the majority of plethodontid species is the male delivery of courtship pheromones: chemical signals that can increase female receptivity (Houck and Arnold, 2003). In one group of plethodontid species, the large eastern Plethodon, this pheromone delivery typically occurs during the tail-straddling walk. In an early study of this courtship behavior, Arnold (1977) proposed that a male assesses the level of receptivity in his mate and then adjusts his courtship behavior accordingly. One common adjustment is for the male to deliver courtship pheromones if the female appears to be reluctant. This delivery occurs while a pair is in tail-straddling walk and the male turns back and “slaps” the female’s snout with his pheromone-producing submandibular mental gland. Following a slap delivery, the male usually turns back and the pair proceeds forward in tail-straddling walk. The number of successive slaps during courtship reflects the level of male persuasion required before spermatophore deposition occurs. Thus, the tail-straddling walk and the slapping mode of pheromone delivery are two highly consistent behaviors among plethodontid species.

However, closer inspection of pheromone delivery behavior reveals that males may not be as finely attuned to a female as previously believed. Observations of many courtships in the Red-Legged Salamander (Plethodon shermani) strongly suggested that some males had individual patterns of pheromone delivery that seemed to be prolonged beyond the point when the female was receptive. These male patterns were observed in the context of
courtship experiments designed to test and document pheromone effects on females (e.g., Rollmann et al., 1999, 2003). In these experiments, hundreds of encounters were staged between male-female pairs. Among the pairs that mated during these staged encounters, we observed that certain males seemed consistently likely to continue slapping, even when the female’s behavior indicated a high level of receptivity (per Arnold, 1977). To investigate this apparent anomaly, we analyzed data from earlier experiments to test the hypothesis that, independent of female response, individual males had a significant degree of consistency in pheromone delivery behavior. Specifically, we compared the number of slaps that a male delivered in each of two different courtship encounters.

MATERIALS AND METHODS

Behavioral Experiments.—Data from experiments on male–female courtship behavior in *P. shermani* were available from earlier studies (conducted in 1996, 1997, 1998, and 2000) that tested whether male courtship pheromones affected female courtship behavior. A complete description of general animal maintenance and of staging and observing courtship behavior is available in Rollmann et al. (1999, 2003) and is summarized briefly here.

Collections of *P. shermani* were made in Macon County, North Carolina (lat. 35°12′30″N; long. 83°12′30″W). Each salamander was housed individually to control for courtship activity. On a trial night when courtships were observed, a male–female pair was placed in a plastic courtship box (31 × 17 × 9 cm). Approximately 40 pairs were observed on each trial night. A pair was defined as entering into courtship when the female stepped over the male’s tail with her forelimbs and the pair moved forward together over the substrate (tail-straddling walk). For the purposes of these pheromone experiments, the mental gland was removed from each male prior to courtship encounters to limit pheromone delivery to experimental treatments. Males lacking a mental gland still enacted the same slapping behavior (LDH, unpublished data). Other variables were recorded for these earlier studies, but here we focused only on the number of times the male slapped (attempted to deliver pheromone to the female).

Statistical Analyses.—To examine the consistency of male courtship behavior, we compiled a data set of males that had two completed courtships that resulted in spermatophore deposition. Laboratory observations of multiply mating males are relatively difficult to obtain; hence, it was necessary to compile a data set that spanned multiple years of experiments (*N* = 50 for years 1997 through 2000). Repetability could not be calculated with only two data points per individual. Consistency of male courtship behavior was analyzed by modeling the number of slaps that each male delivered in a courtship encounter as a function of the number of slaps delivered in a previous courtship encounter. The type of treatment (pheromone or saline control) that had been delivered to a female during an experimental courtship was included as a covariate. As a common starting point for data analysis of counts, the behavioral score data were fitted using a Poisson distribution. For a Poisson distribution, the variance must equal the mean in a condition termed “equivariance,” but this condition usually is too restrictive for count data (Cameron and Trivedi, 1990; Seavy et al., 2003). Model fitting revealed substantial evidence of extra-Poisson variation about the mean (deviance goodness-of-fit test: *χ*<sup>2</sup> = 133, *P* < 0.00001). Thus, our data were analyzed using a quasi-Poisson log-linear regression model that included an extra parameter to model dispersion about the mean. Quasi-maximum likelihood estimation was performed using generalized linear regression modeling in S-Plus 8.1 (TIBCO Spotfire).

The coefficient of determination, *R*<sup>2</sup>, is the percentage of the total variation explained by an explanatory variable in least-squares estimation. Here, we adapt the *R*<sup>2</sup>-calculation for maximum-likelihood estimation such that likelihood *R*<sup>2</sup> is the percentage of total deviance explained by the explanatory variable: likelihood *R*<sup>2</sup> = (total deviance – residual deviance) / total deviance.

RESULTS

One data point (highlighted in Fig. 1) was identified as a potential outlier. Case influence diagnostics (Pearson and deviance residuals) had not identified this data point as particularly problematic; however, omitting this male’s data from the analysis both improved the fit of the model (as shown by residual plots) and demonstrated sufficient change in our consistency estimates to identify this as an unusual and influential data point. Accordingly, we proceeded with our investigation with the outlier omitted and have restricted the scope of inference accordingly. For comparison purposes, analysis with and without this data point is described below.

FIG. 1. The number of pheromone-delivery slaps given by a *Plethodon shermani* male to a female during two courtships. The curve is the estimated log-linear regression model that relates the number of slaps delivered during a previous courtship to the number of slaps delivered by the same male in a subsequent courtship. The potential outlier highlighted at the far right has been identified as an unusual and influential data point.
Analysis including all data revealed a significant effect of previous slapping behavior on subsequent slapping behavior: the estimated mean number of slaps for a male that slapped once in a previous courtship was 6.8, and the mean increased by a factor of 1.35 for each 10-slap increase from the previous courtship (quasi-likelihood t-tests: $t_{43} = 3.1, P = 0.003$; Fig 1). This analysis yielded a likelihood $R^2$-value for previous slapping behavior of 0.15, such that 15% of the total deviance in subsequent (log-transformed) slapping behavior could be explained by previous slapping behavior. In contrast, analysis excluding the influential data point changed the estimated mean number of slaps in a subsequent courtship to 5.9, increasing by a factor of 1.58 for each 10-slap increase from a previous courtship (quasi-likelihood t-tests: $t_{44} = 3.6, P = 0.0008$) and increased the likelihood $R^2$-value of for previous slapping behavior to 0.26. There was no significant evidence that pheromone treatment affected slapping frequency (drop-in-deviance F-test: $F_{2,44} = 0.13$).

DISCUSSION

Our study is one of the few to demonstrate consistency in courtship behaviors of salamanders. In male Red-Legged Salamanders, the consistency between the number of pheromone delivery attempts (“slaps”) in each of two courtships was 26%. Considering that behavioral traits usually are not associated with high levels of repeatability in vertebrates (e.g., Boake, 1989; Michalak, 1996), this between-courtship similarity in male behavior is likely to be behaviorally meaningful. For comparison, a recent meta-analysis found the repeatability estimates (a value highly correlated with our measure of consistency) for courtship behavior in vertebrates to be approximately 0.33 (Bell et al., 2009). However, the repeatability estimates can vary across the behavioral repertoire of a courting individual, (e.g., the calling song of male crickets was more repeatable than their courtship song; Zuk et al., 2008), and the lower consistency estimates observed within our study system may be reflective of this phenomenon. In addition, our consistency estimate could have been influenced by the differences in female receptivity across trials (e.g., the time between courtships a female encountered or the number of prior courtship encounters for the female). At some level, consistency may be lowered when males are encountering females with varying receptivity. Although this variation may have influenced our analysis, the data were not available to account for female receptivity statistically. Regardless, the significant measure of consistency found in our study suggests that, even if a female has received sufficient stimulation and indicates behavioral receptivity, a male still might continue the slapping delivery of pheromones. Such a behavioral conflict between the male and female may reflect the male’s efforts to ensure that the female is in the highest state of behavioral readiness before he begins spermatophore deposition. A similar situation apparently occurs in Montandon’s Newts (Triturus montandon): male courtship behavior (the number of tail-fanning bouts until spermatophore deposition begins) is significantly repeatable (Michalak, 1996). Tail-fanning is a way of transferring waterborne pheromones to the female (see Halliday 1975). Thus, both aquatic and terrestrial male salamanders may benefit by consistency in pheromone delivery because, if a female leaves the male during spermatophore deposition, the male is still committed to completing the deposition (resulting in a reproductive failure).

The interaction between male and females during courtship may be further elucidated by investigating the behavior of P. shermani females. Although useful for many research questions, typical experimental designs do not always offer the opportunity to analyze female variation in mating behavior (Baugh and Ryan, 2009). The P. shermani courtships that we analyzed were from earlier experiments that consisted of data only from “complete” courtships: that is, each courtship continued until the male and female were in tail-straddling walk and the male deposited a spermatophore (the female usually was inseminated). Our current data set also did not have any males that attempted to deliver pheromones a large number of times (e.g., 30–40 slaps). These large slap numbers were common in the data from earlier experiments but only for males that courted to completion a single time. This lack of multiple complete courtship records for males that had high slap rates suggests that, when a female was paired with such an individual, she was more likely to leave that male before sperm transfer could occur. Thus, selection may favor males that deliver an optimal number of slaps because our consistency estimate suggests that slapping behavior is partially genetically determined. Future studies of female P. shermani response to courting males could address the question of whether a female is more likely to leave a male that continues slapping long after the female demonstrates behavioral readiness for spermatophore deposition.

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