The Túngara Frog, *Engystomops pustulosus*, is a small leptodactylid that reproduces in ponds and pools of water and deposits its eggs in foam nests. In the following study the authors compare the body size of amplectant individuals from a population in southwestern Costa Rica, to determine if size-dependent or size-assortative mating occurs in this species. Pictured here is a sexual pair of these frogs in axillary amplexus.

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Is mating in the Túngara Frog (*Engystomops pustulosus*: Leptodactylidae) random or non-random?

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ABSTRACT: Random and non-random mating result from sexual selection, and both can occur across populations of the same species. Body size is a trait commonly used to determine if mating is random or non-random, and for the latter there are two possible outcomes: size-assortative mating and size-dependent mating. Females of the Túngara Frog (*Engystomops putulosus*) from Panama are larger than males, although no correlation in the body size of amplectant individuals has been reported, which suggests random mating. Here, we evaluate whether random or non-random mating (i.e. size-assortative or size-dependent mating) occurs in a population of *E. pustulosus* from southwestern Costa Rica. We measured the body size of amplectant individuals in the field, and did not find a significant difference between the snout–vent length (SVL) of paired males and females or a correlation among the SVLs of all paired individuals. When we considered body size differences among paired males and females, however, we observed size-dependent mating. We suggest that non-random mating must be determined by using more robust analyses, and that a comparison of life-history traits and mating strategies among populations is necessary to determine divergence.

Key Words: Anura, Costa Rica, sexual selection, size-assortative mating, size-dependent mating

RESUMEN: El apareamiento aleatorio y no aleatorio resultan de la selección sexual y ambos pueden ocurrir entre poblaciones de la misma especie. El tamaño del cuerpo es un rasgo comúnmente utilizado para determinar si el apareamiento es aleatorio o no aleatorio, y para el este último hay dos resultados posibles: el apareamiento basado en el tamaño y el apareamiento dependiente del tamaño. Las hembras de la Rana Túngara, (*Engystomops putulosus*) procedentes de Panamá son más grandes que los machos, aunque no se ha reportado una correlación en el tamaño corporal de individuos amplectantes, lo que sugiere un apareamiento al azar. Evaluamos si se produce apareamiento aleatorio o no aleatorio (es decir, apareamiento basado en el tamaño o apareamiento dependiente del tamaño) en una población de *E. pustulosus* del suroeste de Costa Rica. Medimos los tamaños corporales en el campo de individuos amplectantes y no encontramos diferencia significativa en la longitud hocico–cloaca (LHA) de machos y hembras apareados o una correlación entre las LHA de los individuos apareados. Sin embargo, al considerar las diferencias de tamaño corporal entre parejas de machos y hembras, observamos apareamiento dependiente del tamaño.

Sugerimos que el apareamiento no aleatorio debe ser determinado usando un análisis más profundo y que una comparación de los caracteres de historia de vida y estrategias de apareamiento entre poblaciones es necesaria para determinar su divergencia.

Palabras Claves: Anura, apareamiento basado en el tamaño, apareamiento dependiente del tamaño, Costa Rica, selección sexual

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INTRODUCTION

The theory of sexual selection predicts two possible outcomes when mating occurs. Random mating takes place when an individual has the same chance to mate as any other individual in the population, and results from little variation in the perceived quality of mates (Phadke et al., 2012). Conversely, non-random mating occurs when females (usually the selecting sex) more frequently choose mates with specific characteristics, among compatible individuals (Andersson, 1994).

Non-random mating can adopt two forms: assortative mating and dependent mating. Assortative mating occurs when males and females tend to select mates based on a similarity of phenotypic or genotypic traits to themselves, and it can be positive (i.e., similar traits in both mating individuals) or negative (i.e., opposing traits in males and females) (Crespi, 1989; Jiang et al., 2013). Dependent mating is the result of females selecting males based on a specific characteristic, such as suitable body condition, large body size, or age (e.g., old individuals) (Howard, 1988). Both types of non-random mating have been considered powerful evolutionary forces, which increase or decrease genetic relatedness (Robinson et al., 2012) and produce reproductive isolation between divergent populations (Bolnick and Kirkpatrick, 2012).

Non-random mating is a common phenomenon in a wide range of taxa (Jiang et al., 2013), although populations of the same species can vary in mating strategy depending on several factors, such as male density, female preferences, and male-male competition (Arak, 1983; Olson et al., 1986; Tejedo, 1988; Sullivan et al., 1995; Wells, 2007). Body size usually is a good predictor of mate quality and is crucial for many species during intra- or intersexual selection, and thus both size-assortative and size-dependent mating have received substantial attention (Andersson, 1994; Sullivan et al., 1995). In anurans, snout–vent length (SVL) is a widely used measurement of body size (Arak, 1983; Jiang et al., 2013), and many examples of both size-assortative (Briggs, 2008; Fan et al., 2013) and size-dependent (Howard, 1988; Nali et al., 2014) mating have been reported, although significant variation has been observed within and between species (Olson et al., 1986; Wells, 2007).

The Leptodactylidae is a family of Neotropical frogs composed of over 200 species, including the genus *Engystomops* (nine species) (AmphibiaWeb, 2010). Many members of this family build foam nests, and males congregate in ponds and call in choruses (Savage, 2002). Both random (e.g., *Engystomops* [formerly *Physalaemus*] *pustulosus* [Ryan, 1983; Lampert et al., 2006]) and non-random (e.g., *Physalaemus fischeri* [formerly *P. enesefae*] [Tárano and Herrera, 2003] and *Engystomops pustulatus* [Ron et al., 2014]) mating strategies have been reported in this family. *Engystomops pustulosus* is a nocturnal species whose behavior and ecology have been studied extensively (Sexton and Ortleb, 1966; Ryan, 1985; Dawson and Ryan, 2012). Although this species is distributed from Mexico to Venezuela and Trinidad and Tobago, on both the Atlantic and Pacific versants (Savage, 2002), the vast majority of studies regarding sexual selection have been conducted in Panama, where Ryan (1983) studied a population on Isla Barro Colorado and determined that females were larger than males, but found no correlation in the SVL of amplectant males and females.

In Costa Rica, *E. pustulosus* is a common species along the northwestern and southern portions of Pacific versant, where it breeds from May to November (Savage, 2002). Adult males (SVL 25–34 mm) call from stationary positions in both temporary and permanent small bodies of water, which can be located in natural or disturbed areas. Several males often call from the same pond, and calling behavior can vary according to the number of conspecifics (Ryan, 1985). Adult females (SVL 26–35 mm) are less numerous (e.g., 5 males : 1 female [Ryan, 1983]) and only visit ponds to find a mate. Information on the mating strategies of populations in Costa Rica, however, is scarce.

Both assortative mating and dependent mating can significantly impact the direction of selection, by producing a number of effects on a population that can result in reproductive isolation and, eventually, in speciation (Crespi, 1989; Jiang et al., 2013). Therefore, comparing the mating strategy across populations of the same species is crucial to understanding the evolutionary pathway of each population. Here, our goal is to evaluate whether size-assortative or size-dependent mating occurs in a population of *E. pustulosus* from southwestern Costa Rica, and to compare the mating system across populations of this species from Costa Rica and Panama.

MATERIALS AND METHODS

Study Site

We conducted our study in Golfito, Provincia de Puntarenas, Costa Rica (8°38'42.42"N, 83°10'27.41"W; datum WGS 84; elev. 10 m), a humid (average annual precipitation = 5,000 mm) and hot (average annual maximum temperature = 31°C) coastal area (Maldonado, 2005). Golfito was constructed as a strip of buildings in an area of tropical rainforest, and the abrupt topography of the region has allowed for the presence of wildlife within the environs of the town. Today, *Engystomops pustulosus* frequently is encountered in ponds or other bodies of water within the forest and along the town's roadways.

Data Collection

We collected data from 3 to 7 October of 2000. We searched for frogs in small bodies of water along the roads of the town from 1830 to 2300 h. We focused on amplectant pairs, and determined their sex by inspecting for the presence of brown nuptial pads on the thumbs and a vocal sac in males (Savage, 2002). We measured the SVL of individuals with a ruler to the nearest 1 mm. We searched for the frogs along a 2 km trail and sampled each pond only once, and released all individuals at their point of capture.

Data Analysis

To determine whether random or non-random mating occurs, first we examined whether males and females differed in their SVL, and then correlated the SVLs of amplectant individuals. In addition, we calculated the difference in the body sizes of females and males (difference = female SVL minus male SVL) and determined whether females of similar size (i.e., difference in body sizes of females and males between –1 and 1 mm; this range representing the measurement error), larger (i.e., difference < –1 mm) or smaller (i.e., difference > 1 mm) than males selected larger mates. As was done by Ryan (1983) on Panamanian populations, we used a contingency table to compare categories of male SVL (Small = < 29 mm; Medium = 29–31 mm; Large = > 31 mm) and categories of difference in body sizes of paired males and females.

RESULTS

We measured a total of 34 amplectant pairs. We did not detect a significant difference between the SVL of paired males and females ($t_{66} = -1.25$, $P = 0.217$, $\bar{x} \pm$ SD: males = 30.15 ± 2.19 mm, females = 30.79 ± 2.09 mm). We observed 16 amplectant pairs with similar SVLs, 12 amplectant pairs where females were larger than males, and only six amplectant pairs where males were larger than females (Fig. 1). We found no significant difference in the number of amplectant pairs according to the categories of difference in body sizes of males and females ($\chi^2 = 4.47$, $df = 2$, $P = 0.107$).

We did not find a correlation among the SVL of all paired individuals ($r = -0.086$, $P = 0.628$, $n = 34$; Fig. 2), which suggests random mating based on body size. Nonetheless, we observed more medium-sized males in amplexus than small and large ones ($\chi^2 = 8.18$, $df = 2$, $P = 0.017$, Table 1). Small males only mated with females

larger than themselves, large males most often mated with smaller females, and medium-sized most frequently were paired with females of a similar size (Fisher's Exact Test $P < 0.001$, Table 1).

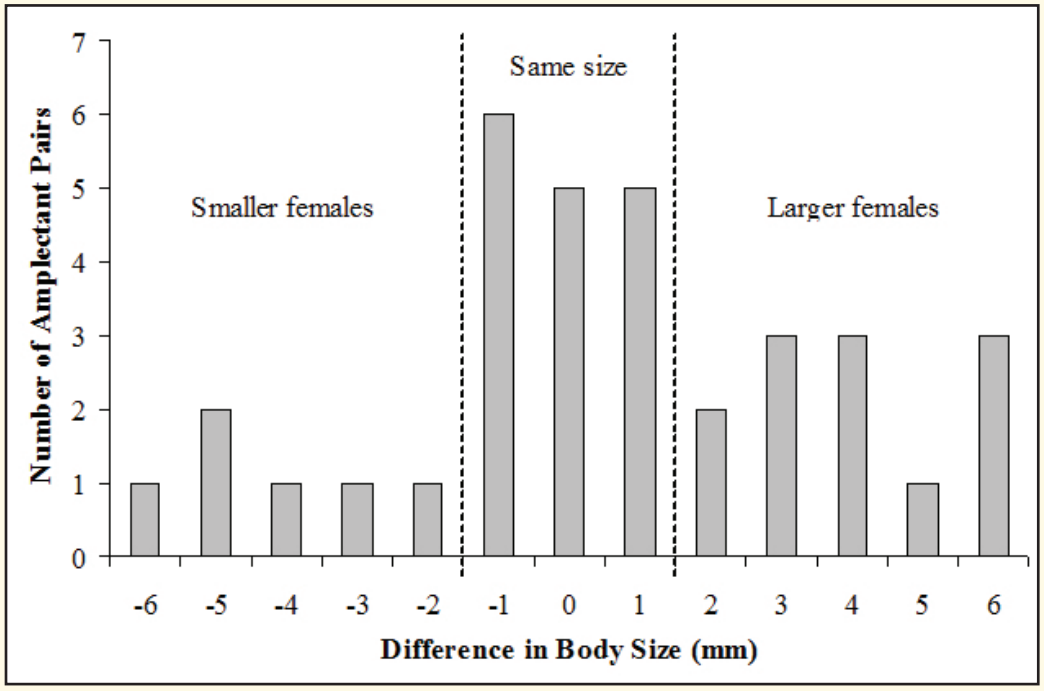

Fig. 1. Distribution of body size differences (female SVL minus male SVL) among 34 amplectant pairs of *Engystomops pustulosus* in Golfito, Provincia de Puntarenas, Costa Rica.

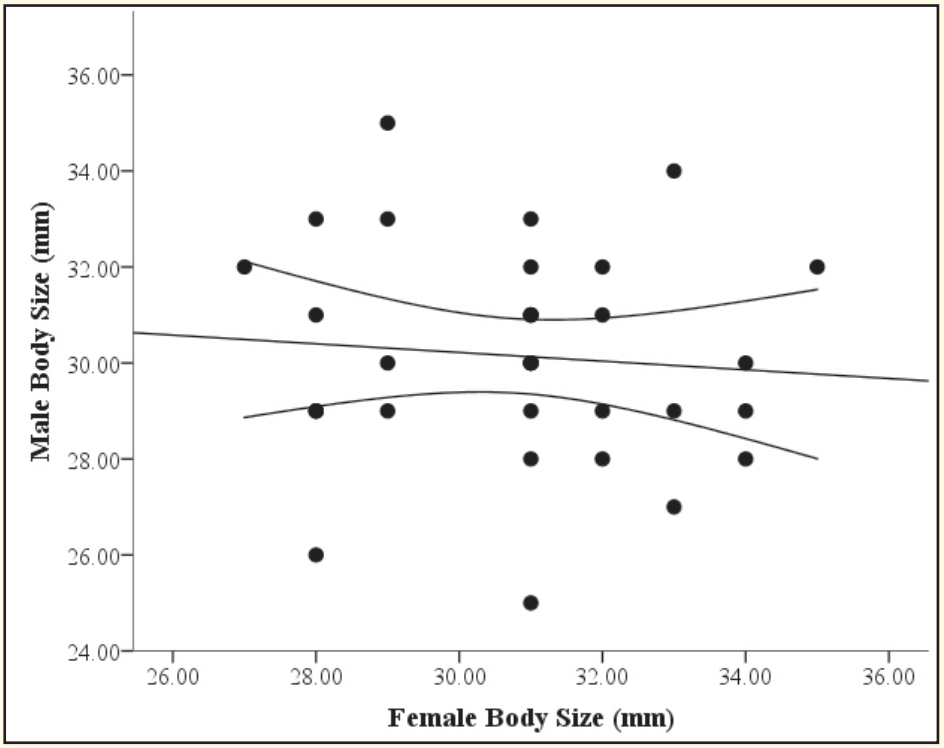

Fig. 2. Pearson correlation of female and male body sizes of *Engystomops pustulosus* captured during amplexus ($P = 0.628$) in Golfito, Provincia de Puntarenas, Costa Rica.

Table 1. Number of matings according to body size differences of amplectant individuals and male size categories in *Engystomops pustulosus* from Golfito, Provincia de Puntarenas, Costa Rica.

Male Body Size Categories	Body Size Difference of Amplectant Individuals			Total Number
	Smaller Females	Same Size	Larger Females	
Small	0	0	6	6
Medium	1	13	5	19
Large	5	3	1	9
Totals	**6**	**16**	**12**	**34**

DISCUSSION

Two main findings emerged from our data: (1) We found some divergence in the mating strategy between populations of *Engystomops pustulosus* from Panama and Costa Rica; we compared our results in the mating strategy exhibited by individuals from a southern Pacific population in Costa Rican with those of Ryan (1983) for a population in central Panama; and (2) an overall assortment does not occur in our study population based on a simple correlation of the body sizes of amplectant individuals; however, non-random mating apparently occurs when considering categories of body size difference among males and females. Based on our results, size-dependent (not size-assortative) mating might be occurring.

Ryan (1983) found a difference in the size of unmated males and females, with the latter being larger, and proved that random assortment occurred even when size categories were considered. Conversely, our results show no difference in SVL between paired males and females, and that most amplectant females selected males similar to their own size or smaller. These findings apparently contradict those of Ryan (1983), and suggest that females in our study population choose their mates based on size. Our results highlight the fact that a comparison of life-history traits and mating strategies among populations of the same species is necessary to determine whether divergence is occurring. Genetic divergence across populations of *E. pustulosus* has been quantified, with populations from northwestern Costa Rica differing from those from southwestern Costa Rica (there is a gap in the distribution of *E. pustulosus*, as no specimens have been recorded from the central Pacific of Costa Rica) and Panama (Pröhl et al., 2006). Although the populations of *E. pustulosus* in southwestern Costa Rica and the one studied by Ryan (1983) in Panama have been shown to be closely related, sexual selection seemingly acts in different ways.

We did not measure non-amplectant males, and thus we cannot establish the full range of male body sizes in the population nor determine the female preferences. The range of body sizes of the males we measured (25–35 mm) reflects that reported in the literature for sexually mature individuals (Savage, 2002), suggesting that our sample is representative of the distribution of male SVLs in the population. Therefore, it is reasonable to consider that females are, in fact, selecting males according to size and this selection could be the result of the reduction of potential adverse effects during amplexus. An optimal size difference hypothesis (Licht, 1976) predicts that females will select males that are not too large to interfere with their free movements or too small that would be unable to fertilize most eggs. Apparently this is the case with *E. pustulosus*, as Ryan (1983) found that an increasing difference in size between mating individuals increases the number of undeveloped eggs.

In addition to body size, multiple factors have been shown to influence female preferences and male reproductive success in *E. pustulosus*, such as the frequency and complexity of calls, the number of nights of chorus attendance, and the body condition (Green, 1990; Marler and Ryan, 1996). Moreover, some of these factors can be correlated; for example, large males produce low frequency and more elaborate calls that seem to be preferred by females (Ryan, 1985), although as call elaboration increases their relative attractiveness decreases (Akre et al., 2011). If the costs of large male size decrease mating success, then selection would not favor larger males (Zhu et al., 2016) and medium-sized males would have an advantage over large males.

We explored mate choice as a potential explanation of this mating strategy in a population of *E. pustulosus* from southwestern Costa Rica. A larger sample size of mating pairs, SVL measurements for unmated individuals,

and a measure of reproductive success (e.g., clutch size and hatching success), will help elucidate the effects of this non-random mating in *E. pustulosus*. Our results, however, increase our knowledge of sexual selection in this species, and underscore the need to conduct comparative studies at the population level.

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