

LACK OF SIZE-ASSORTATIVE MATING IN A WIDESPREAD TREEFROG: *PSEUDACRIS CRUCIFER* IN IOWA

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ABSTRACT: The Spring Peeper (*Pseudacris crucifer*) is a small treefrog native to much of Central and Eastern North America. Here, we tested if Spring Peepers exhibit size-assortative mating—non-random mate pairings based on body size—from a population in eastern Iowa. We captured 75 amplexant pairs of Spring Peepers during the 2021 breeding season, measured their body sizes, and marked them for future recognition. We found that while sexual-size dimorphism exists between males and females, there was no evidence for size-assortative mating among amplexant pairs. Our study contributes to other recent work indicating that size-assortative mating is rare among most anurans, and that previous findings of this phenomenon in frogs should be interpreted with caution.

Key Words: frog, size-assortative mating, Iowa, Spring Peeper, amphibian

INTRODUCTION

Assortative mating is a premating isolation mechanism, which can play a powerful role in the evolutionary trajectory of a species because individuals will base mating decisions non-randomly (Kopp et al. 2018). Size-assortative mating (SAM) is a behavior in which individuals display preferences for larger mates, thus sorting by body size when breeding (Shine et al. 2001). Thus, SAM is an important driver of sexual selection which promotes variation, evolutionary change, and can even lead to speciation (Janicke et al. 2019). In anuran amphibians, larger individuals of both sexes generally have breeding advantages over their smaller counterparts. Larger male frogs, for example, have greater access to females by holding onto larger territories (e.g., Howard 1978, Reichert and Gerhardt 2011). Larger females tend to have greater fecundity measures, such as larger clutch sizes, relative to smaller females (e.g., Shine 1979, Monnet and Cherry 2002). The benefits of greater body size suggest that at least some aspects of mate choice in frogs are related to body size, and many previous authors have found support for such positive SAM in frogs (e.g., Márquez and Tejedo 1990).

In a meta-analysis, Jiang et al. (2013) found that SAM was widespread in most animals, including anurans,

which they linked to maximizing reproductive success. However, a more recent meta-analysis by Green (2019) found that this assumption did not hold for anurans and called into question the validity of previous findings supporting positive SAM. In fact, Green (2019) reanalyzed the anuran data from Jiang et al. (2013) and found a non-significant relationship, which he attributed to a statistical phenomenon known as Simpson's Paradox where results differ if data are divided into subpopulations (Galipaud et al. 2015). Consequently, the positive publication bias supporting a strong correlation of SAM within species was likely derived from authors pooling data inappropriately, rather than subdividing data sets by allochronic or allopatric independence (Green 2019). For example, SAM may not occur within one population but when multiple populations are pooled together a positive relationship may emerge. Alternatively, SAM may not occur on one night within a breeding season, but when multiple nights are pooled together a positive relationship may emerge (Rios Moura et al. 2021). This aspect of analyzing SAM in anuran studies has produced a publication bias where positive relationships were more likely to be reported (Green 2019, Rios Moura et al. 2021).

Pseudacris [Hyla] crucifer (Spring Peeper) is a small treefrog in the family Hylidae that is distributed across

much of central and eastern USA, including Canada. Sexual-size dimorphism (SSD) is present in the Spring Peeper, where females exhibit larger body sizes relative to males (Dodd 2013). In fact, per mm increase in SVL, female Spring Peepers can increase the number of eggs per clutch anywhere from 8 to 21% (New York: Oplinger 1966), though this relationship may exhibit geographic variation (e.g., Arkansas: Trauth et al. 1990). The SSD in Spring Peepers suggests that some components of mating outcomes could be related to body size, thus we set out to examine SAM in Spring Peepers during a single breeding season from a single population in eastern Iowa while explicitly controlling for Simpson's Paradox.

METHODS

Study site

Karl W. Behrens Memorial Ponds and Woodland State Preserve is a 11.7 ha site located in Toddville, Linn County, eastern Iowa. From 1895 to 2023, Linn County received an average annual precipitation of 69.25 cm (\pm 9.02 cm) and exhibited an average annual temperature of 8.13 °C (\pm 0.36 °C) (NOAA 2023). The site was deeded to the Nature Conservancy by Karl W. Behrens in 1977, then dedicated as a state preserve in 1982 (Herzberg and Pearson 2001). The sandy soil of the site stems from Wisconsin Glacier sediment deposits made about 12,000 years ago (Herzberg and Pearson 2001). The soil at the site has led to a variety of unique habitats for the region, including oak forest, open prairie, and vernal wetlands. The site has four vernal pools of varying size and depth, which are partly covered by forest canopy but still typically dry up in the summer, usually by August. We focused our sampling efforts for Spring Peepers at these pools.

Amphibian sampling

We sampled Spring Peepers during the breeding season (based on active choruses) using aquatic funnel traps (Frabill, Pure Fishing, Inc., Jackson, WI) across three vernal pools. Based on the filling of ponds from precipitation events, we were able to run two trapping sessions in 2021 of equal length (Session 1: 10–19 March; Session 2: 23 March–1 April). Traps were spaced approximately 5 m apart and distributed along the edge of each pool with the number of traps deployed per pool dependent upon its size. Traps were checked for frogs every 24 hrs between 0700–1200 hr. We note that all pairs were found in amplexus inside the traps, which were placed in individual bags for processing. We measured the snout-vent length (SVL) of all captured frogs with digital calipers to the nearest 0.01 mm and weight to the nearest 0.1 g using a spring scale (Pesola, Switzerland). We tagged frogs with visible implant elastomers (VIE) using a day-specific cohort mark. Frogs were then released at their site of capture. Recaptured frogs were identified with ultraviolet light to visualize VIE tags and were excluded from the following statistical analyses.

Statistical analysis

We organized and analyzed data using Microsoft Excel 2016 (Redmond, WA) and R (R Core Team 2022) with the RStudio interface (Posit Team 2022). We checked variables for normality by visualizing QQ plots and conducting Shapiro-Wilks Tests, and ran non-parametric tests if the data were not normally distributed. To test for sexual dimorphism, we compared the mean size of males and females using two-sample t-tests. To explore SAM, we conducted correlations between body size metrics of am-

plectant pairs using the Pearson method. To ensure our results did not violate Simpson's Paradox, we ran regression analyses on the body size data by subdividing the amplexant pairs by individual sampling nights. These preliminary results indicated that our data were temporally independent, thus we felt confident to pool data across all sampling nights (Green 2019). Mean values are presented with \pm 1 standard deviation and statistical significance was recognized at $P < 0.05$.

RESULTS

Over 20 trap nights, we captured 75 amplexant pairs of Spring Peepers for a total of 75 males and 75 females. For amplexant pairs, the means of female SVL (27.43 ± 1.20 mm, $n = 75$) and weight (1.40 ± 0.25 g, $n = 75$) were significantly larger than the means of male SVL (23.52 ± 1.50 mm, $n = 75$) and weight (0.94 ± 0.17 g, $n = 75$) ($t = 17.8$, $df = 142.3$, $P < 0.001$; $W = 5,409$, $P < 0.001$) (Fig 1A-B). For these amplexant pairs, we found no relationship between both body size metrics, for either SVL ($R^2 = 0.00000007$, $t = -0.01$, $df = 73$, $P = 0.99$) or weight ($R^2 = 0.029$, $t = 1.47$, $df = 73$, $P = 0.15$) (Fig 1C-D). We also captured 11 males (mean SVL: 24.70 ± 1.66 mm; mean weight: 1.15 ± 0.29 g) and six females (mean SVL: 28.10 ± 1.48 mm; mean weight: 1.85 ± 0.36 g) not in amplexus. In five situations where a single female was found in a trap with at least two males, females were found in amplexus with the largest male twice and with the smallest male three times. In a single situation where a male was found with two females, the smaller female was the one found in amplexus with the male.

DISCUSSION

From a large sample of Spring Peepers, we detected sexual-size dimorphism in both body length and weight, which we attribute to larger females having a fitness advantage of greater fecundity (Shine 1979, Monnet and Cherry 2002). Despite this clear size discrepancy and its potential advantage, the frogs captured in traps were paired randomly with respect to their mate's body size. Most previous studies examining size-based mating decisions in Spring Peepers also found no evidence for positive SAM (e.g., Gatz 1981, Forester and Czarnowsky 1985, Stewart 2013). Gatz (1981) however, examined just eight amplexant pairs in an analysis of SAM. Forester and Czarnowsky (1985) also did not detect positive SAM, despite having a robust sample size ($n = 182$ pairs), but we note that they pooled data across three years without checking for SAM within years (i.e., Green 2019). These previous studies were conducted in Ohio and Maryland, which represent divergent genetic lineages within the "Northern" clade of the Spring Peeper: the "Interior" and "Eastern" mtDNA lineages, respectively (Austin et al. 2002, 2004, Cairns et al. 2021). Unpublished data from a hybrid zone between the "Interior" and "Eastern" mtDNA lineages in Canada, nevertheless, found positive SAM using just 12 amplexant pairs, but this analysis was based on samples pooled across five sites that included both mtDNA lineages and hybrid populations (Hudson 2010). Furthermore, a follow-up study from the same region using a larger sample ($n = 28$ pairs)—while still pooling data across populations and years—did not find positive SAM for body size (Stewart 2013). When considered together with our data from Iowa, which is from the "West" mtDNA lineage of the "Western" clade, we suggest that SAM does not exist in Spring Peepers, at least within these three lineages and

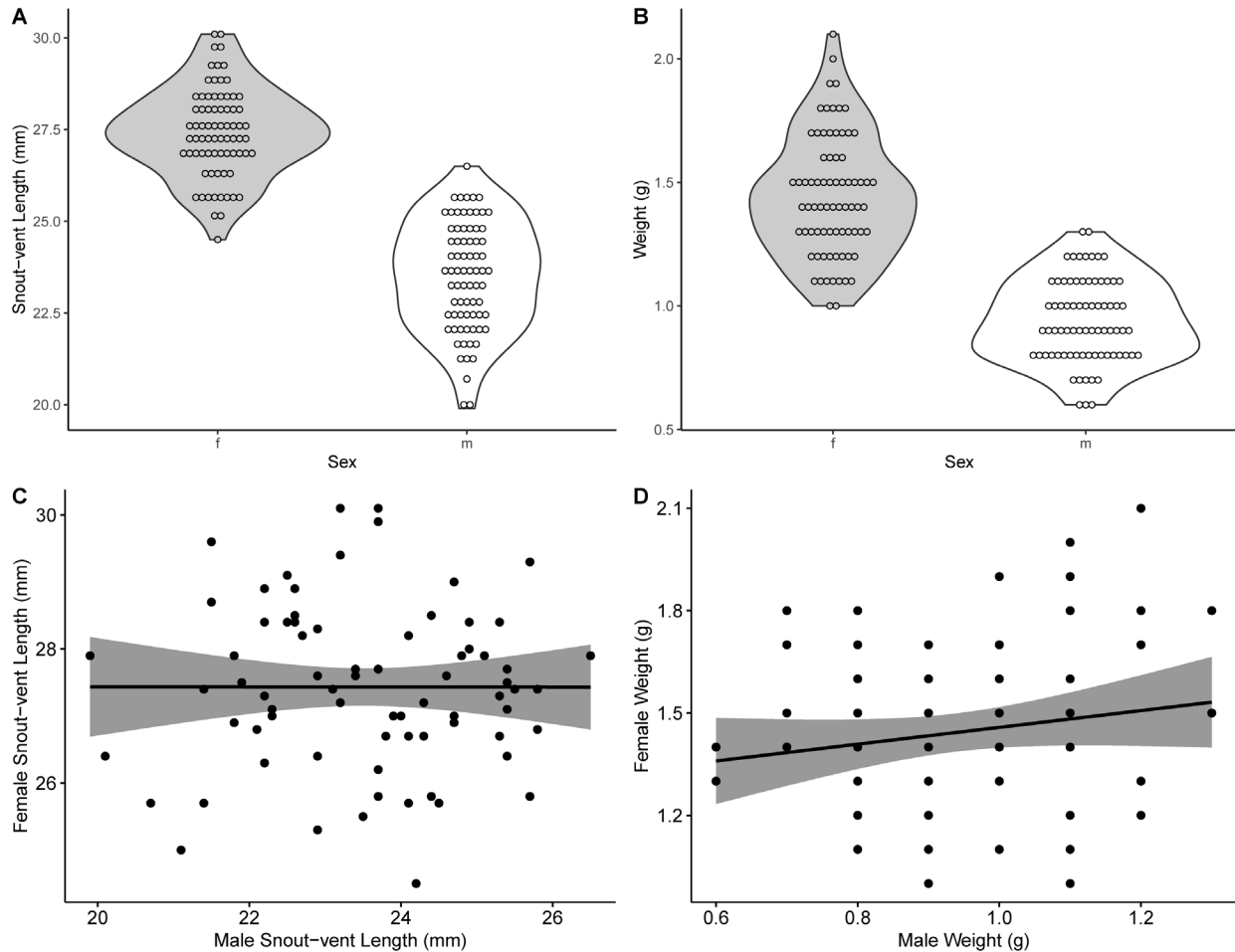


Figure 1. Body size relationships between male ($n = 75$) and female ($n = 75$) Spring Peepers (*Pseudacris crucifer*) in amplexus from Toddville, Linn County, Iowa, USA. Violin plots of snout-vent length and weight (A-B); Scatterplots with lines of best fit and 95% confidence intervals (grey shading) showing the relationships between amplexant pairs for snout-vent length and weight (C-D).

perhaps hybrid populations (Stewart 2013), but populations from the “Southern” clade, or its three mtDNA lineages, have not yet been examined (Cairns et al. 2021). In our study, amplexant pairs were assumed to have formed before they were found in traps as the duration of amplexus in some anurans can last up to 84.9 hrs (e.g., Howard 1980). However, it is possible that specific pairings were an artifact of limited mate choices inside traps. Nevertheless, Murphy and Gerhardt (1996) found female Hylidae, when stimulated under artificial conditions, to discriminate among mates to the same degree as they would in natural conditions.

Within anurans, fertilization is not always associated with body size, and therefore measurement of only amplexant pairs may be a false positive for SAM studies (Galipaud et al. 2015, Green 2019, Rios Moura et al. 2021). Some studies that tracked courtship to fertilization in anurans showed that even if size preferences are present, they may ultimately be unimportant with respect to who successfully fertilizes a clutch (e.g., Fan et al. 2013). Given that SAM does not appear to be affecting mate choice in Spring Peepers, then other traits must be playing more prominent roles, such as the quality of male advertisement calls (Forester and Czarrowsky 1985, Forester and Lykens 1986, Lykens et al. 1989, Sullivan and

Hinshaw 1990). Specifically, several studies have shown that rapid repetition, strong amplitude, and low-frequency calls appear to strongly influence a female Spring Peeper’s preference in a mate (Forester and Czarrowsky 1985, Forester and Lykens 1986, Lykens and Forester 1987, Lykens et al. 1989, Sullivan and Hinshaw 1990). Forester and Czarrowsky (1985) even determined that call frequency in males scaled with body size, yet still found body size to be independent of amplexant pairs. The phenological timing of when different-sized mates arrive at a breeding pool, or even imbalanced sex ratios (Vojar et al. 2015), can also influence the pattern of SAM detected (Dittrich et al. 2018). Quality of perch sites, also, has been shown to influence mating decisions in Spring Peepers by enhancing the conspicuousness of the male’s call, and the presence of satellite males in calling congregations can even impact amplexant pairings (Forester and Czarrowsky 1985, Forester and Lykens 1986). Other work has shown that intra-sexual competition can favor larger males who mate more frequently because they win aggressive interactions or occupy the highest-quality habitats (Taborsky et al. 2009, 2014). Some research in Spring Peepers has shown that non-amplexant males were smaller than those in amplexus (Fellers 1979, Gatz 1981). Ultimately, mating outcomes in Spring Peepers

do not appear to assort by body sizes in Iowa, Canada, Ohio, or Maryland. Fruitful avenues of future research for mating decisions in Spring Peepers include exploring the presence of satellite males, perch site variation of males, phenological timing of breeding arrival, and the quality of male calls, all of which could be tracked to fertilization success within and across lineages.

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