Comparative analysis reveals assortative mate preferences in darters independent of sympatry and sex

Yseult Héjja-Brichard[1], Julien P Renoult[2], Tamra C Mendelson[1]

1 Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, Maryland, United States; 2 CEFE, Univ Montpellier, CNRS, EPHE, Univ Paul-Valery Montpellier, Montpellier, Occitanie, France

Abstract

A preference for mating with conspecifics over heterospecifics is fundamental to the maintenance of species diversity in sexually reproducing organisms. This type of positive assortative mating results in sexual isolation, a reduction in gene flow between species due to differences in mate choice. Sexual isolation is often stronger between closely related sympatric species as compared to allopatric species, because of processes such as reinforcement. In animals, this pattern has been demonstrated across the genus *Drosophila*, however, most empirical evidence for stronger species-level assortative mating in sympatry comes from studies of two or only a few species. In addition, traditional theories of sexual selection suggest that because reproduction is more costly to females, they should be the choosier sex. This prediction was supported in *Drosophila,* but it remains unclear whether this is a general trend. We therefore conducted a comparative analysis of mate preferences in sympatric and allopatric species of darters (genus *Etheostoma*). We performed a meta-analysis of 16 studies, encompassing 21 species. As expected, we found stronger preferences for conspecifics over heterospecifics across studies and species. Contrary to studies in *Drosophila*, however, we did not find an effect of sympatry or sex on the strength of preference for conspecifics. We offer several testable hypotheses to explain the variation we observed in the strength of assortative preference.

Keywords: assortative mating preferences; sexual isolation; *Etheostoma*; mate preference; speciation

Introduction

As evolutionary biology continues to explore the mechanisms of speciation, the processes driving reproductive isolation remain a central focus of study. One important reproductive barrier is sexual isolation, a reduction in gene flow due to differences in mate choice. Sexual isolation is a consequence of assortative mating resulting from a preference for conspecific mates (Jiang et al., 2013; Kopp et al., 2018). Preference for conspecific over closely related heterospecific mates can evolve as a result of divergent natural or sexual selection in geographically isolated populations, and it can be strengthened in sympatric populations due to processes such as reinforcement (Dobzhansky, 1940; Liou & Price, 1994); (Servedio & Noor, 2003). Understanding the evolutionary mechanisms driving the evolution of preference for conspecific mates is an active area of research in the field of speciation.

In 1989, (Coyne & Orr, 1989, 1997) made the important discovery that prezygotic barriers in *Drosophila* were twice as strong in sympatric species than in allopatric species. This geographic pattern was not true for postzygotic barriers (hybrid inviability and hybrid sterility). Yukilevich (2012) later confirmed that this pattern in *Drosophila* is a result of mate choice and not gametic incompatibility. (Lemmon, 2009) found the same pattern at a smaller scale in two frog species of the genus *Pseudacris*. Females from populations in which the two species were sympatric more strongly preferred conspecific signals, compared with allopatric females, and this preference was stronger when the conspecific signal was the sympatric one (Lemmon, 2009). Comparative studies in plants also support a pattern of greater assortative mating in sympatric species, though (Hopkins, 2013) points out ways in which those studies could be strengthened.

A geographic pattern of stronger preference for conspecific mates in sympatric compared to allopatric populations can arise for a number of reasons. One is a process of reinforcement when selection against hybrids in sympatry favors increased preference for conspecific mates (Servedio & Noor, 2003). Another is a process of differential fusion, if geographic overlap only occurs when species have accumulated a sufficient degree of conspecific preference prior to secondary contact (Noor, 1999). A last process arises from species extinction or species sorting whereby costly sexual interaction can dramatically reduce the growth of one species leading to its extinction (Kyogoku & Wheatcroft, 2020). Although the original interpretation of Coyne and Orr’s (1989) results pointed to reinforcement as explaining the pattern of greater sexual isolation in sympatry, more recent analyses suggest that differential fusion and extinction rates might also contribute to a pattern of stronger sexual isolation in sympatry (Matute & Cooper, 2021). Thus, a stronger preference for conspecific mates in populations that are sympatric with a close congener is an important pattern that can both support and generate hypotheses about the evolutionary mechanisms driving sexual isolation.

Another important result of broad comparative studies in *Drosophila* is that of (Yukilevich & Peterson, 2019), who found that for sympatric species pairs, female *Drosophila* had stronger preferences for conspecific males than males did for females, whereas no sex difference in preference was found in allopatric species. That result is consistent with classic sexual selection theory (Andersson, 2004; Trivers, 1972), that females invest more than males in reproduction and thus represent the choosier sex. A greater strength of preference for conspecific mates in females of sympatric populations thus could suggest two things. Mating with a heterospecific in sympatry might be more costly for females than males, leading to reinforcement of females more so than male preferences; or, female preferences evolve faster than male preferences in allopatry, and they are necessary for maintaining species boundaries upon secondary contact.

Here, we focused on darters (genus *Etheostoma*) to perform a comparative analysis of preference for conspecific mates ("assortative preference") and to explore the role of geographic overlap and sex in predicting those preferences. Darters are a large clade of North American freshwater fishes characterized by elaborate secondary sexual traits (Page & Burr, 2011). Mate preference and mate choice have been investigated in several pairs of species of darters. Studies find that species demonstrate a varying degree of preference for conspecific over heterospecific mates (Martin & Mendelson, 2013; Williams & Mendelson, 2013; Mendelson et al., 2018) and that assortative preference and assortative mate choice is present in both females (Williams & Mendelson, 2010, 2011; Roberts et al., 2017) and males (Ciccotto et al., 2013; Zhou et al., 2015; Martin & Mendelson, 2016; Moran et al., 2017; Roberts & Mendelson, 2017; Moran & Fuller, 2018), depending on which heterospecific is presented. Moreover, hybridization has been documented in many darter species (Bossu & Near, 2013; Keck & Near, 2009), which could provide the substrate for reinforcement (i.e., hybrids with reduced fitness), making this clade a good system to examine geographic and sex-specific patterns of mate choice at the genus level.

Two studies of darters have tested explicitly for stronger assortative preference in sympatric populations in one or a small number of species pairs. Moran and Fuller (2018) compared male choice and aggressive behaviors in a small number of closely related species (*Etheostoma caeruleum* and members of the *Ceasia* species complex). They found that both male preferences for conspecific females and aggressive behaviors towards conspecific males were stronger in populations that were sympatric with the congener. Roberts and Mendelson (2020) measured the strength of assortative preference in allopatric and sympatric populations of two darter species (*Etheostoma zonale* and *E. barrenense*). They also found a stronger preference for conspecific mates in sympatric populations, but only in females, which contrasts the results of Moran and Fuller (2018), who found assortative preference only in males.

In this paper, we performed a phylogenetically informed meta-analysis to determine the extent to which increased preference for conspecific mates in sympatry is present in *Etheostoma*. We hypothesize that differences among species in preference for conspecifics are driven by geographic relationships, predicting that sympatric species will have a stronger preference. We also compared the strength of preference for conspecifics between males and females. Most sexual selection theory predicts that females will be choosier (Andersson, 1994; Darwin, 1871; Trivers, 1972 but see Edward & Chapman, 2011), and Yukilevich and Peterson's (2019) results in *Drosophila* are consistent with that prediction. However, given abundant evidence of male mate choice in darters, and the contrasting results of two studies of assortative preference, the relative importance of male and female mate choice in sexual isolation, in general, remains an open question.

Material and Methods

Inclusion criteria

We conducted our meta-analysis on studies that investigated mate preference in *Etheostoma* in a comparable manner over the past twelve years (2010-2023). We included 12 published papers (indicated with an asterisk in the reference section) and five unpublished datasets, encompassing 21 species of *Etheostoma* distributed across the phylogeny of the genus (Near et al., 2011), arranged in 14 different pairs of species.

All studies used a dichotomous mate preference paradigm where individuals do not have physical access to one another. The main measure of preference is the time the focal fish spent in an association zone adjacent to either a conspecific or a heterospecific individual of the opposite sex (Figure 1). Most studies allowed only visual cues, with focal and stimulus fish separated into different tanks. Two studies (O'Rourke & Mendelson, 2010 and Barber & Mendelson, unpublished) used partitions to separate focal and stimuli fish that were not water-tight, thus potentially allowing exchanges of olfactory cues.

This conservative choice of experimental design removes aggressive physical interactions among members of the same sex as an explanation for the behavior of the focal fish. It also emphasizes mate preference rather than mate choice, as choice is better measured by allowing physical access to potential mates (as in e.g. Moran et al., 2017; Zhou et al., 2015). Studies in our analysis were conducted by different lead authors in different physical lab spaces but at the same university, using similar protocols. This similarity in design is well suited for meta-analyses, which often suffer from discrepancies in experimental design across studies (Dougherty & Shuker, 2015).

Effect size calculation

All included studies measured the time spent in the association zones. Sample sizes, means and standard deviations were extracted from each paper and when those variables were not available, we contacted corresponding authors to obtain the raw data. The effect size was calculated as a Pearson's correlation coefficient *r* of times spent in conspecific association zones minus heterospecific association zones for each tested species and sex of each study (Equation 1):

$$r = \frac{d}{\sqrt{d^2 + \frac{1}{p*(1-p)}}}, \text{ (Equation 1)}$$

$$\text{with } d = \frac{\bar{\mu}_{sample_C} - \bar{\mu}_{sample_H}}{\sigma_{pooled}},\ p = \frac{N_{sample_C}}{N_{sample_C} + N_{sample_H}},$$

$$\text{and } \sigma_{pooled} = \frac{\sigma_{sample_C}{}^2 * (N_{sample_C} - 1) + \sigma_{sample_H}{}^2 * (N_{sample_H} - 1)}{N_{sample_C} + N_{sample_H} - 2}$$

where $\overline{\mu}_{sample}$ and σ_{sample} correspond to the mean and standard deviation of the time spent with conspecifics ($\overline{\mu}_{sample_C}$) and with heterospecifics ($\overline{\mu}_{sample_H}$), N_{sample} corresponds to the sample size of tested individuals, which is identical for both conspecifics and heterospecifics in our case. We did not Z-transform our correlation coefficient as this can affect the accuracy of statistical models (see Janicke et al., 2019 for a similar decision).

Moderators and their rationale

We sought to determine which factors might influence the strength of preference for conspecifics over heterospecifics, as represented by the effect size. We selected two 'natural' factors: geography and sex of the tested individual, and three 'experimental' factors: the size of the association zones, stimulus type, and recording duration times.

Geography: We predicted the geographic relationship between the two species in a pair to influence the strength of preference for conspecifics, with a stronger preference in sympatric species. For each study, we determined whether species pairs were allopatric or sympatric (Etnier & Starnes, 1994; Lee et al., 1981; Page, 1983) and included this variable as a moderator. Some species pairs consist of both allopatric and sympatric populations (i.e., incomplete range overlap); pairs were scored according to the population of origin.

Sex: Although classical sexual selection theory predicts a stronger preference in females, some studies have found the opposite pattern, with a stronger preference for conspecifics in males compared to females (e.g., Mendelson et al., 2018; Moran & Fuller, 2018). Our dataset includes as many male-focal individuals as females, which allows us to compare assortative preferences between the sexes.

Experimental factors: Previous work showed that experimental design impacts mating preference outcomes (Dougherty & Shuker, 2015). Moreover, response to video playbacks compared to live fish varies with at least one of the tested species (Roberts et al., 2017) and could potentially be affected by the sex of the focal individual. We thus included three moderators to reflect the variability in experimental setups. Namely, we included the size of the association zones (5 or 10 cm), the stimulus type (behaving fish, motorised model, fish animation, video playbacks), and recording duration times (5, 10, 15, or 20 min) as experimental factors in our model. We predict that a larger association zone and longer recording duration will result in stronger effect sizes as more data are included. We expect some differences between species due to variation in stimulus type (e.g., live versus video playback).

Phylogeny

To control for the non-independence of the strength of preference due to a shared evolutionary ancestry that varies between species pairs, we included phylogenetic information in our statistical models, using Near and colleagues' (2011) phylogeny. Their phylogeny is based on the cytochrome b mitochondrial gene and two nuclear gene sequences, the S7 ribosomal protein (first intron) and the recombination activating gene-1 (RAG1, exon 3). We retrieved the data file (Nexus format) from TreeBase. We pruned the phylogenetic tree with the drop.tip function from the ape R package (Paradis & Schliep,

2019) to keep only our 21 species of interest. In the case where several individuals per species were available, we kept the individual from the closest geographical population to the population studied in our meta-analysis. The resulting tree (Figure 4) was converted into a matrix of phylogenetic distance that was included in our meta-regression models.

Statistical analyses

All statistical analyses were carried out in R version 4.2.0 (R Core Team, 2022). We used the package metafor (Viechtbauer, 2010) to perform the meta-analysis modelling. To determine the overall mean effect size, we ran a first multi-level meta-analysis model fitted via restricted maximum likelihood ("REML") estimation with the function rma.mv. We included study identity as a random effect to account for the non-independence of effect sizes. We removed species as an additional random effect as this variable explained 0% of the variance and removing it slightly decreased the AIC score of the model. Phylogeny was included in all our models as a variance-covariance matrix estimated from the phylogenetic tree. To assess the respective influence of our different moderators (i.e. explanatory factors) on the mean effect size, we ran meta-regression models for each moderator separately (function rma.mv with the 'mods' parameter). We calculated the level of heterogeneity across all effect sizes using the I^2 statistic to determine how generalizable our findings are (Higgins et al., 2003). Script for statistical analyses and data are available on OSF [https://osf.io/hnf8m/].

Results

In total, we extracted 68 effect sizes from 16 studies investigating 21 focal species of darters. This includes 32 effect sizes for males and 36 for females, 42 for allopatric and 22 for sympatric populations.

We found no publication bias in our dataset as shown by a roughly symmetrical funnel plot (Figure 2). This was further confirmed by Egger's regression test ($Z = -0.2687$, $p = 0.7881$).

Our multi-level meta-analysis model revealed an overall effect size of medium strength ($r = 0.3213$, $p = 0.0006$, CI = 0.1382 – 0.5044), corresponding to positive preferences for conspecifics. A negative effect size would correspond to preferences biased towards heterospecifics, and an effect size not different from 0 to an absence of assortative mate preference. The total heterogeneity across effect sizes (I^2) amounts to 30.08% (14.15% from the study identity and 15.93% from phylogeny).

Our investigation of the respective influence of our moderators with separate meta-regression models showed that none of them impacted variation in effect sizes. We found no overall difference in preference strength between males and females, no difference between sympatric and allopatric populations, and no effect of any of the experimental factors that we included (size of the association zones, stimulus type, and recording duration times). However, given the highly unbalanced sample sizes for those experimental factors, conclusions should be carefully drawn. The results of our meta-regression models are summarised in Table 1.

Discussion

Our meta-analysis of 16 datasets encompassing 21 species of darters revealed a positive mean effect size of preference for conspecific mates when using a dichotomous paradigm based on visual cues. This result suggests that most species included in our analysis prefer conspecifics of the opposite sex over heterospecifics. Our investigation of potential moderators of the strength of this preference suggests that neither geographic relationships (i.e., sympatry versus allopatry), nor sex, nor any of the three experimental factors (association zone size, stimulus type, and recording time durations) predictably influence the strength of preference for conspecifics.

An overall preference for conspecifics is consistent with previous meta-analyses conducted at higher taxonomic levels (Janicke et al., 2019; Jiang et al., 2013). These analyses investigated assortative mating across species of birds, insects, fishes, amphibians, gastropods, and crustaceans and found similar trends for overall positive assortative mating. Janicke et al (2013) investigated the strength of assortative mating in 307 species in 130 families across these taxa and found an overall effect size of 0.36, independent of species richness. They confirmed the results of Jiang et al (2013), who obtained an overall effect size of 0.26 across 254 species in five animal phyla, though that study did not correct for phylogenetic non-independence. If we assume that assortative preference is a logical precursor to assortative mating, then our results showing assortative preference across *Etheostoma* demonstrate overall assortative mating at the genus level, with an effect size of 0.32.

We further investigated the effect of geographic overlap and sex on the strength of assortative preference, as both variables are thought to modulate assortative mating. Based on the findings of Coyne and Orr (1989) in *Drosophila,* whose estimation of prezygotic isolation for sympatric pairs was at least twice as large as for allopatric pairs, as well as other studies of single species pairs showing a similar pattern (Höbel & Gerhardt, 2003; Pauers & Grudnowski, 2022), we predicted stronger assortative preference in sympatric species of *Etheostoma*. Our statistical analyses did not support this prediction. Rather, out of the four sympatric populations included in the meta-analysis, two showed a stronger preference for conspecifics (*E. zonale* and *E. barrenense*), one had no assortative preference (*E. flabellare*) and one, *E. olmstedi*, showed unexpectedly disassortative preference, with individuals from sympatric populations showing a stronger preference for heterospecifics (Figure S1). Interestingly, the allopatric populations of *E. olmstedi* that were also included in the analysis did not show such a reverse pattern but rather a stronger preference for conspecifics (Figure S2).

This variation across species in the relationship between geographic overlap and assortative preference might suggest that species, and populations within species, differ in the costs and benefits of mating with heterospecifics. The classic cost of mating with heterospecifics is a reduction in offspring fitness, and the strength of this postzygotic barrier is likely to vary across species pairs. Alternatively, this variation might reflect differences in the extent of syntopy between nominally sympatric species. For example, *E. barrenense* and *E. zonale*, a species pair in the analysis in which both species exhibit strong assortative preference, are notably syntopic where they overlap. *E. barrenense* is more strongly associated with bedrock substrate and *E. zonale* with vegetation (Greenberg, 1991; Hlohowskyj & Wissing, 1986), yet both are commonly found and collected together over bedrock or coarse gravel (pers obs).

Given evidence of reduced survival in the hybrids of this species pair (Williams & Mendelson, 2014), assortative preference between these species may have arisen due to reinforcement (see Roberts & Mendelson, 2020). In contrast, *E. olmstedi* and *E. flabellare*, between which preference is either absent or disassortative, are known to exhibit distinct habitat and foraging preferences (Greenberg, 1991; Proulx, 2014) and may represent micro-allopatric species. Thus, the conditions that predict stronger assortative preference in sympatry, including the opportunity for maladaptive hybridization, may not hold for this species pair.

As such, our results do not support the prediction of overall greater assortative preference in sympatric populations or species. Rather, they highlight variation among species that can help explain when and why assortative preferences evolve, for example, due to variation in the costs and benefits of interspecific mating, or in the extent of true geographic overlap. Additional factors that might affect the strength of assortative preference include the presence or absence of nuptial coloration. Assortative mating based on color pattern differences has been shown in a number of animal taxa, including Midas cichlid fish (Elmer et al., 2009), *Heliconius* butterflies (Jiggins et al., 2001), and strawberry poison frogs (e.g., Summers et al., 1999). Notably, two species in our analysis that show strong assortative preference are strikingly colorful, and previous studies show that both males and females of these species preferentially associate with conspecific color patterns (Williams & Mendelson, 2011, 2013). In contrast, the species in our analysis that lacks assortative preference (*E. flabellare*), and the species for which assortative preference varies across populations (*E. olmstedi*), are largely achromatic. Adding additional darter species that vary in chromatism therefore could allow us to test whether the relationship between assortative preference and geographic overlap is predicted by the presence of nuptial coloration. Alternatively, species might differ in temporal patterns of mating, with some mating earlier or later in the season. Like micro-allopatry, this type of temporal reproductive isolation precludes maladaptive hybridization and thus precludes selection against hybrids leading to increased assortative preference in sympatry. A broader survey of species could allow us to include these and other additional factors, with multiple species pairs representing each factor, and thus evaluate whether they modulate the relationship between geography and assortative preference.

In addition to geography, we investigated the effect of sex on the strength of preference for conspecifics. Classical sexual selection theory predicts that females will be choosier than males, as the cost of investment is expected to be skewed towards females (Andersson, 2004; Trivers, 1972). In our meta-analysis, sex did not have a significant effect on the strength of preference for conspecifics. Although counter to conventional sexual selection theory, this result is consistent with the natural history of darters. Male darters invest considerably in reproduction, with energetic courtship displays, nuptial coloration, and in some species, paternal care (e.g. Kelly et al., 2012; Mendelson et al., 2018). Indeed, similar to poison frogs (Summers et al., 1997), darter species that exhibit paternal care are mostly achromatic, suggesting a potential trade-off in energy allocation for reproduction.

The absence of an effect of sex on assortative preference is also consistent with several previous studies in darters. For example, assortative preference was stronger in males than in females when secondary contact was simulated in artificial streams for allopatric species of darters in the earliest stages of divergence (Mendelson et al., 2018). Similarly, for the darter species *E. caeruleum* and members of the *Ceasia* (*E. spectabile*) species complex, only males of sympatric species showed greater preference for conspecifics (Moran & Fuller, 2018). Yet in other studies, female darters exhibited stronger assortative preference than males, as in *E. nigripinne* (O'Rourke & Mendelson, 2010). Thus, the absence of an effect of sex on the strength of preference seems to accurately reflect the diversity of reproductive behaviors and preferences in this genus. Including even more species in the analysis could allow us to identify groups of species (e.g., subgenera) in which males are choosier than females or vice versa. Such a pattern could further elucidate the factors or conditions driving sex differences in assortative preference.

Beyond the biological implications of our results, we also sought to determine whether experimental factors could influence effect sizes when comparing multiple studies. In terms of the type of stimuli, i.e., whether stimulus fish were live, model (dummy), video playback, or computer animation, we found that it did not affect the strength of preference for conspecific stimuli. This result is an important validation of the use of artificial stimuli in mate choice research (Chouinard-Thuly et al., 2017; Powell & Rosenthal, 2017), in that artificial stimuli appear to be as useful as live animals in detecting mate preference. Artificial stimuli allow researchers to replace and reduce the number of live animals in experiments, as encouraged by ethical animal use protocols. Results of Williams & Mendelson (2011) corroborate our finding, showing equivalent responses to live and dummy fish in *Etheostoma zonal*e and *E. barrenense*. Roberts and colleagues (2017) found equivalent responses to live and video playback stimuli in *E. zonale*, but not in *E. barrenense*. Thus the type of stimulus may have some effect on the strength of preference, but this appears to be type- and species-specific. We note that most studies in our analysis used live stimuli. Nonetheless, we continue to explore the efficacy of various stimulus types in testing mate preference in darters.

Although we did not expect a significant effect of recording time duration, we found a tendency for longer durations to have smaller effect sizes. It might thus be important to keep the duration of observation short (i.e. under 10 min) as the expression of preference may begin to taper after a few minutes as the focal individual loses interest. Besides mean durations in association zones, additional measures of fish preference could provide important information. Additional behavioral measures might include the fish's head orientation or line of sight, and pursuit behaviors, to quantify interest in the presented stimuli. For instance, two studies in darters reported glass jabbing behavior as a measure of a female's mating interest and of a male's aggressive behavior (Soudry et al., 2020; Williams & Mendelson, 2013). One study also reports the number of times a fish visits an area (Soudry et al., 2020), which could indicate exploratory differences between species or sexes that may reflect preference.

Finally, the last experimental factor that varied between studies was the size of the association zones. In their study comparing several pairs of darter species, Mendelson and colleagues (2018) reported results for zone sizes of both 5 and 10 cm. We found no difference between 5 and 10 cm in terms of effect size neither in their study (t = -0.6319, df = 53.624, p = 0.5301) nor in our meta-analysis (data for 5cm was included in the meta-analysis, but including the data for 10cm instead made no difference for effect sizes). However, effect sizes tended to be larger for wider association zones, which is logical, since a bigger area of the tank can be occupied for a longer period of time. To avoid inflating results with larger association zones, we recommend adjusting the size of the association zone to reflect the visual acuity of the tested species (Caves et al., 2017).

Conclusion

Conducting a meta-analysis of dichotomous mate preference trials in darter fish (*Etheostoma*), we found an overall effect size of medium strength, indicating a preference for conspecific over heterospecific mates across the genus, with no significant difference between males and females. Comparing sympatric and allopatric populations, we found no effect of geographic overlap on the strength of preference. Our results suggest that the link between assortative mating and geography is not as straightforward as might be predicted from hypotheses of reinforcement, and the link between assortative mating and sex is not as straightforward as classical interpretations of sexual selection might suggest. We postulate several factors that could modulate the strength of preference for conspecifics in the genus *Etheostoma* and explain the lack of evidence for an overall effect of geography and sex. Our results thus highlight the complexity of assortative mating and the need for further study. Given the diversity of the genus, *Etheostoma* is a great system to investigate such questions.

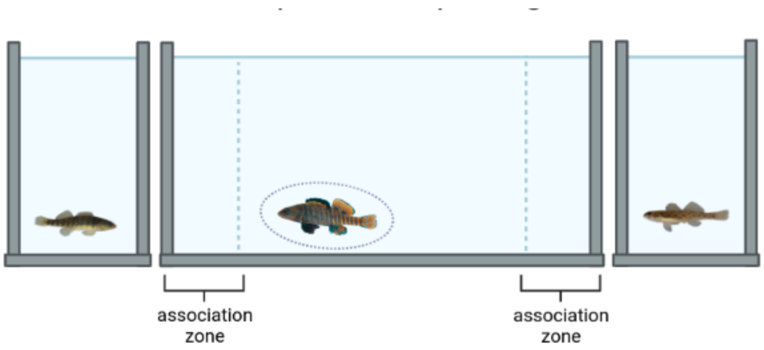
Table

Table 1: Table with moderators: Q_M, *p*-value, mean, and CIs estimated in separate models for each moderator.

Moderator	Q_M	*p*-value	Mean	Sample size	95% CIs
Sex of the focal individual	0.5113	0.4746	F: 0.3018	36	0.1104 - 0.4932
			M: 0.3544	32	0.1572 - 0.5516
Allopatry vs sympatry	1.4724	0.2250	A: 0.2552	42	0.0515 - 0.4588
			S: 0.3731	26	0.1410 - 0.6052
Size of the association zone	0.4915	0.4832	5cm: 0.2914	59	0.0908 - 0.4920
			10cm: 0.4002	9	0.1093 - 0.6912
Stimulus type	2.4512	0.4842	Live: 0.3037	59	0.1152 - 0.4923
			Video: 0.2498	3	-0.1702 - 0.6698
			Motorised: 0.5531	4	0.2057 - 0.9006
			Animation: 0.2406	2	-0.3434 - 0.8246
Recording times	1.3574	0.7156	5min: 0.3991	4	-0.1316 - 0.9297
			10min: 0.3789	1	-0.2631 - 1.0209
			15min: 0.4397	19	0.2066 - 0.6728
			20min: 0.2686	44	0.0618 - 0.4755

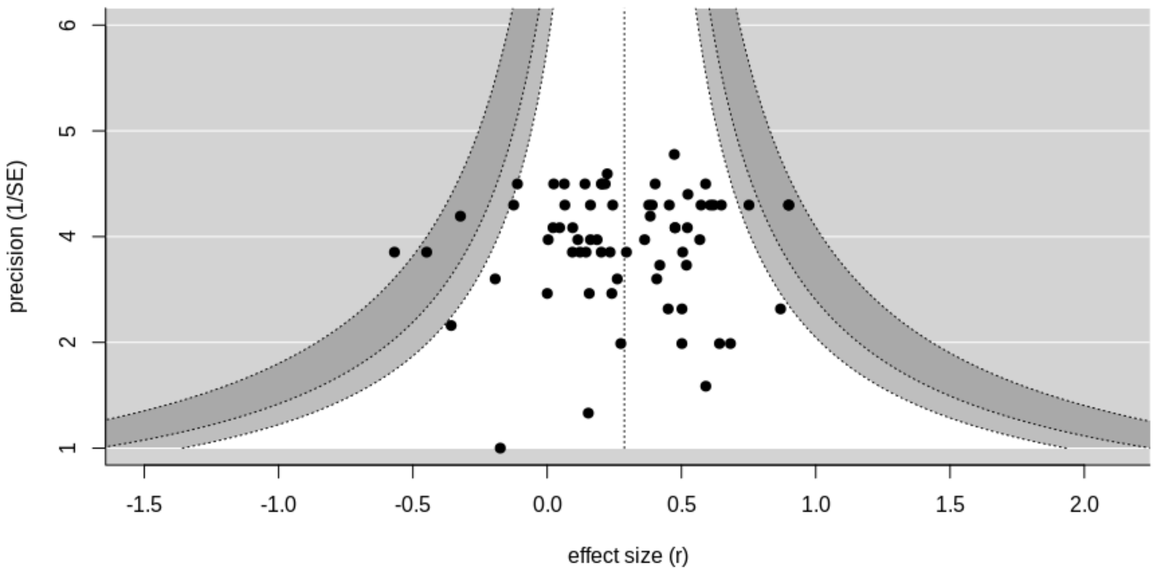
Figures

Figure 1: Mate preference experimental paradigm



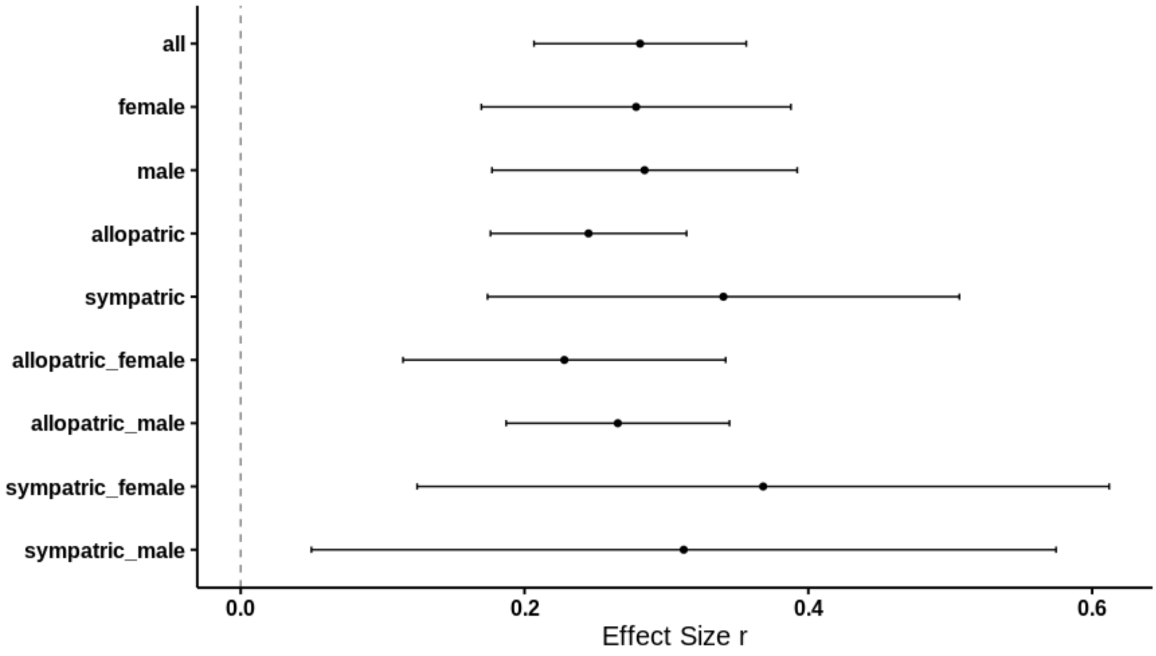
Legend: Illustration of a dichotomous mate preference paradigm. The main measure is the time that the focal fish (circled) spends in either association zone adjacent to either a conspecific or a heterospecific individual of the opposite sex. The exact design varies between studies as mate options can be live fish, motorised models, videos or computer animations displayed on a monitor. Created with BioRender.

Figure 2: Funnel plot to test for publication bias



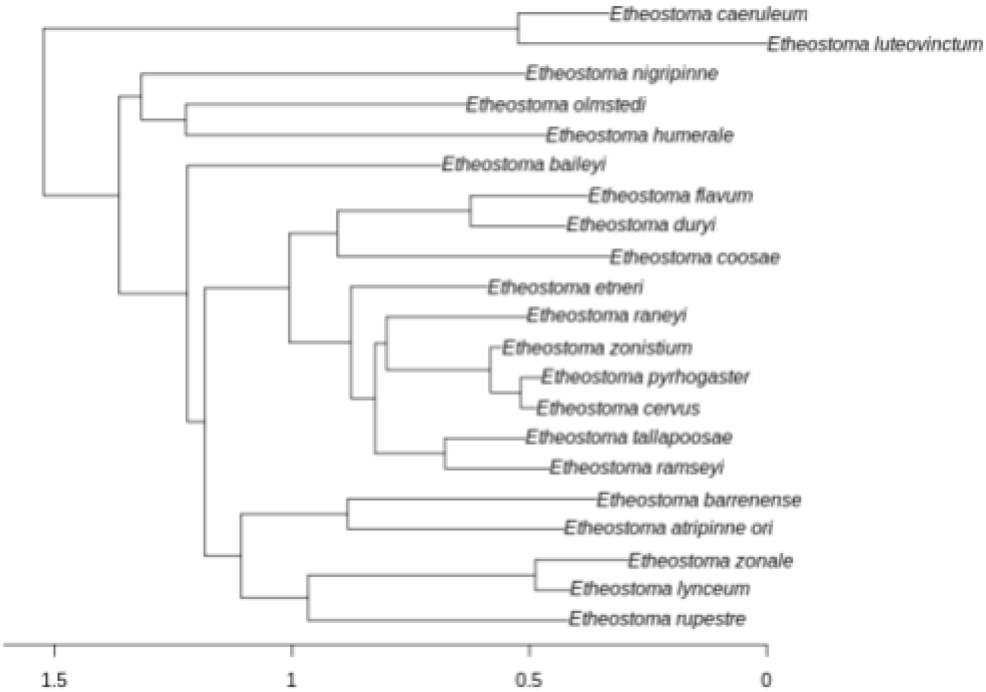
Legend: The funnel plot is roughly symmetrical, indicating no publication bias, which was confirmed by Egger's regression test: Z = -0.2687, p = 0.7881.

Figure 3: Forest plot of effect sizes



Legend: Forest plot showing mean effect sizes (± CIs) calculated using Equation 1 of the main moderators: sex and geographic relationship, as well as an average of all the moderators included in the analysis.

Figure 4: Phylogeny of the focal species included in the meta-analysis



Legend: Phylogenetic tree of the 21 focal species included in the meta-analysis, based on the phylogeny of Near et al. (2011). Note that E. humerale *corresponds to our* E. flabellare *(collected from the Potomac River, an Atlantic slope drainage).*

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The star (*) indicates papers included in the meta-analysis.

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Supplementary material

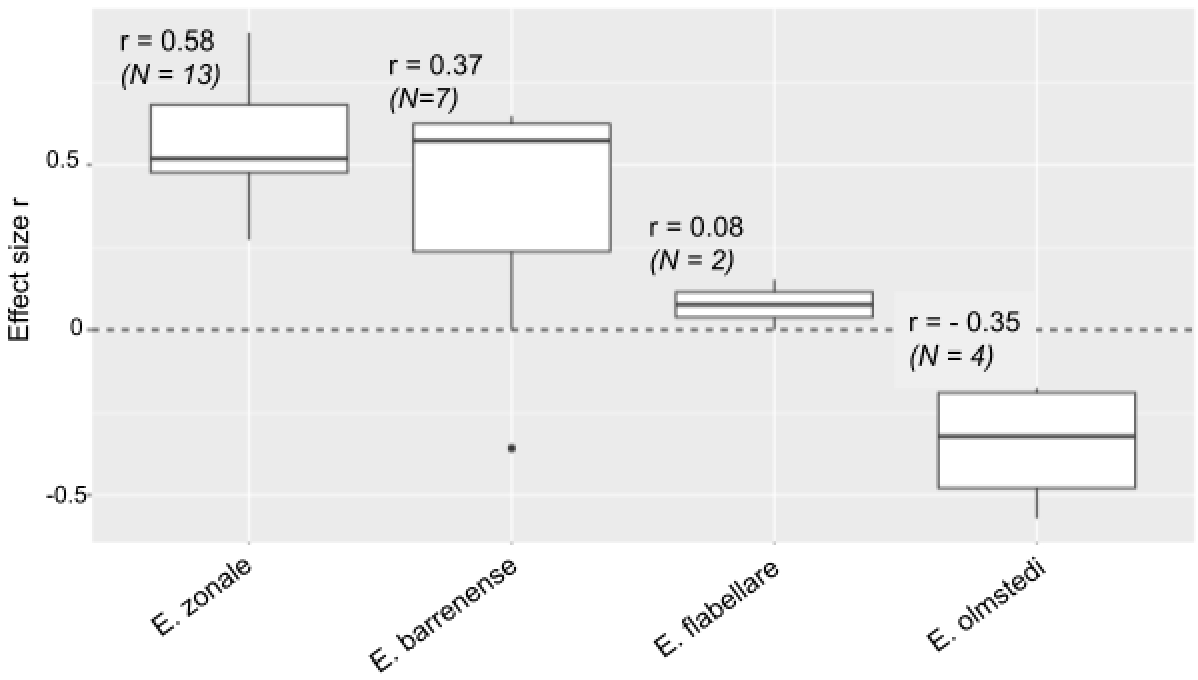

Figure S1: Effect sizes for the sympatric populations included in the meta-analysis (descending order). r gives the effect size value for the sympatric population of the species. N indicate the number of effect sizes (i.e. number of experiments) that were included per population.

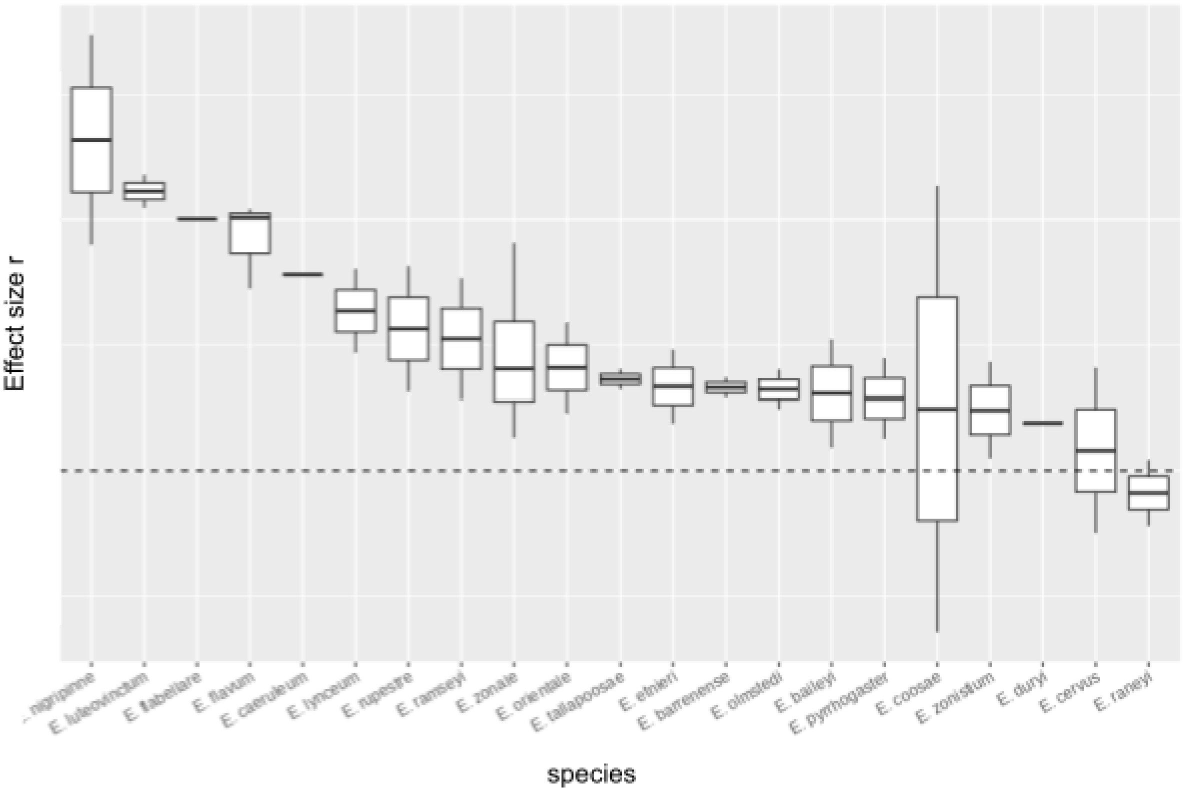

Figure S2: Effect sizes for the allopatric populations included in the meta-analysis (alphabetical order).