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SHORT COMMUNICATION

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Little to no inbreeding depression in a tapeworm with mixed mating

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Abstract

Meta-studies on hermaphrodites have found a negative relationship between primary selfing rates and levels of inbreeding depression (ID) and, thus, generally support purging in inbred systems. However, in plants, high among-taxa variance in ID results in no difference in the mean ID between outcrossing and mixed-mating taxa. Selective interference likely explains high ID among mixed-mating taxa, whereas low levels of ID among mixed-mating taxa are not as stressed. Among animal hermaphrodites, primarily molluscs, there are little data on mixed-mating systems. To fill a taxonomic and mating system gap, we tested for ID in a mixed-mating tapeworm, *Oochoristica javaensis*. We provide a direct estimate of ID across infection of an intermediate host by comparing selfing rates at two life history stages. We found little to no evidence for ID, and the level of ID falls in line with what is reported for highly selfing species even though *O. javaensis* has mixed mating. We discuss this result within the context of kin mating in *O. javaensis*. Our results emphasize that primary selfing rates alone may be insufficient to classify the inbreeding history in all species when testing for a relationship to ID. Mixed-mating taxa, and possibly some outcrossing taxa, may exhibit low levels of ID if biparental inbreeding is also driving purging. We advocate that ID studies report estimates of inbreeding history (e.g. F_{IS} or identity disequilibrium) from nature-derived adult samples to provide context rather than relying on primary selfing rates alone.

KEYWORDS
cestode, inbreeding, inbreeding depression, odds ratio, *Oochoristica javaensis*, selfing rate

1 | INTRODUCTION

Self-mating in hermaphroditic organisms can have important fitness consequences in a single generation such that selfed (inbred) offspring could have reduced fitness compared to that of outcrossed offspring, that is inbreeding depression (ID; Charlesworth & Willis, 2009). Despite the possible immediate negative fitness consequences, theoretical and empirical work show that repeated bouts of self-mating, or more generally, a history of inbreeding, can purge deleterious alleles if the reduction in fitness is caused by large effect, largely recessive alleles, especially those affecting early-expressed traits (Charlesworth and Willis 2009 and references therein). Purging, though, is not as effective for overdominant polymorphisms or deleterious mutations of weak effects affecting late-expressed traits (Charlesworth & Willis, 2009). Nonetheless, because of the potential for purging and the possible feedback for purging to drive the evolution of self-mating (Lande & Schemske, 1985; Porcher & Lande, 2016), there has been interest in testing for an association

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between selfing rates and the degree of ID (Escobar et al., 2011; Husband & Schemske, 1996; Winn et al., 2011). In particular, a negative relationship between the amounts of inbreeding and ID is expected when ID is reduced via homozygous exposure of recessive or partially recessive deleterious mutations (Latta & Ritland, 1994).

Three meta-studies (two in plants and one in animals) have examined the relationship between primary selfing rates (i.e. the proportion of selfed progeny, typically measured at the earliest possible life history stage) and levels of ID across multiple hermaphroditic species (Escobar et al., 2011; Husband & Schemske, 1996; Winn et al., 2011). Broadly speaking, all three studies found a significant negative relationship between taxa selfing rates (an implicit proxy for past inbreeding) and levels of ID. Nonetheless, these studies highlight notable findings, limitations and/or future directions that indicate there are still gaps in our understanding of the evolutionary interplay between inbred mating systems and purging. For example, all three studies showed extensive variation in levels of ID (δ) among outcrossing taxa (selfing rates, s, ≤ 0.2) and mixed-mating taxa ($0.2 < s < 0.8$) such that the range of δ estimates extend from values around 0.1 to close to 1. Selfing taxa ($s \geq 0.8$) had a more confined range with δ values just below 0 to less than 0.5 (see figure 2 in Husband and Schemske 1996; figures 2 and 6 in Winn et al. 2011; figure 4A in Escobar et al. 2011). Interestingly, Winn et al. (2011) found that there was no difference in mean δ between the outcrossing and mixed-mating taxa; a result, they argued, that was not expected if mixed mating represented an evolutionary transition between selfing and outcrossing. They argued purging in some mixed-mating taxa is likely precluded by selective interference, that is ID is so high that no selfed offspring survive to reproduce, and as such, there can be no purging (theoretical treatment in Lande, Schemske, and Schultz 1994). As support, Winn et al. (2011) highlight taxa-specific biological features that may explain high δ in some mixed-mating taxa (e.g. longevity of gymnosperms leads to high mutation rates, which in turn promotes selective interference).

A few limitations are noted in the animal-based study (Escobar et al., 2011). Although taxa-specific traits may explain variation in δ, Escobar et al. (2011) also question "the reality of mixed mating in animals" noting that the five taxa in their study classified as mixed mating may have unreliable selfing rate estimates due to various issues. In particular, progeny-array data to estimate primary selfing rates are more difficult to obtain in animals than plants. As such, many animal studies rely on the inbreeding equilibrium relationship $F = s/(2 - s)$ to estimate selfing rates, where s is a constant selfing rate across generations and F is the equilibrium inbreeding often estimated with the deviation from Hardy–Weinberg estimator F_{IS} (Jarne & Auld, 2006; Jarne & David, 2008). However, Escobar et al. (2011) note that estimates of F_{IS}, and hence s, could be overestimated due to technical (e.g. null alleles) or biological factors (e.g. the Wahlund effect). Moreover, Escobar et al. (2011) highlight that in animal studies, an underestimated value of δ is often obtained because selfing cannot be prevented in outcrossing treatments; they term this measure "apparent ID." Lastly, the conclusions of Escobar et al. (2011) are largely based on a single group of molluscs (12 basommatophoran molluscs, three other molluscans, one cestode and one branchiopoda; their figure 4a). Indeed, Escobar et al. (2011) call for additional studies from understudied animal clades. For instance, despite species estimates upwards of 130,000 (Strona & Fattorini, 2014), the predominantly hermaphroditic parasitic flatworms (Neodermata: trematodes, cestodes and monogenes) have received little attention regarding the estimation of either primary selfing rates or ID (Benesh, Weinreich, Kalbe, & Milinski, 2014; Criscione, 2016; Detwiler, Caballero, & Criscione, 2017).

To fill a taxonomic gap and mating system gap, we provide a study of ID in a mixed-mating tapeworm, *Oochoristica javaensis*. Adults of this parasite can be found infecting Mediterranean geckos (*Hemidactylus turcicus*) in the southern United States (Criscione & Font, 2001a, 2001b, 2001c). Early diploid larval stages (oncospheres) develop in the maternal tapeworm's terminal segments (proglottids), get released with host faeces and then will be ingested by an intermediate host. The natural intermediate host is unknown although the flour beetle *Tribolium castaneum* is a suitable host in the laboratory (Criscione & Font, 2001b; Detwiler & Criscione, 2011). In the coelom of the beetle, a juvenile stage, generically termed a metacestode (Chervy, 2002; Conn, 1985), develops. The life cycle perpetuates when infected beetles are consumed by the gecko. A recent study estimated individual maternal tapeworm selfing rates via progeny-array data of metacestode genotypes and thus after the passage through an intermediate host (Detwiler et al., 2017). They found that individual selfing rates were highly correlated (inverse power relationship) to the number of tapeworms within a host, that is the infection intensity (Detwiler et al., 2017). Using the distribution of parasites among hosts and assuming random reproductive success, Detwiler et al. (2017) estimated the population selfing rate to be 30.6% (average across five subpopulations using GLMM estimates; figure 2 in Detwiler et al. (2017)). Hence, *O. javaensis* has a mixed-mating system as estimated at the metacestode stage.

Our goal here was to ascertain whether selfing rate estimates obtained from the genotypes of oncospheres would be much higher than previous estimates from the metacestode stage as this would be evidence for ID upon passage through the intermediate host. Our estimate of δ differs from the more common apparent δ measures found in animal hermaphrodite studies (Escobar et al., 2011) in that we used a direct measure of δ based on the survivorship of selfed individuals from oncosphere to metacestode stage (i.e. a comparison of the selfing rates from the two life history stages; Ritland (1990)).

2 | MATERIALS AND METHODS

2.1 | Sample collection

Mediterranean geckos were collected in College Station, Texas, USA. Details of sampling locations and collection protocols were described previously (Detwiler & Criscione, 2011, 2014; Detwiler et al., 2017). Briefly, cestodes were still alive upon dissection. Maternal tapeworms were separated, and then, parasite offspring (oncospheres) were collected directly from a maternal tapeworm by separating

the last 4–8 gravid proglottids. Gravid proglottids were then broken apart to tease out oncospheres, which were subsequently pooled for individual worms. From this pool of oncospheres of a single maternal tapeworm, we created two groups. One group was stored in 70% ethanol and was to be directly genotyped. The other group was fed to beetle intermediate hosts (Criscione & Font, 2001b; Detwiler & Criscione, 2011). After 20 days, developed metacestodes were collected from beetle hosts and stored in 70% ethanol prior to genotyping (Detwiler et al., 2017). Microsatellite genotyping was used to determine whether an individual offspring (oncospheres or metacestodes) was the result of a self-mating or an outcrossing event.

2.2 | DNA extraction and microsatellite genotyping

DNA from adults and metacestodes was extracted following protocols described in Detwiler and Criscione (2011), whereas DNA from oncospheres was extracted using the method reported in Beltran, Galinier, Allienne, and Boissier (2008). Likewise, PCR (polymerase chain reaction) of adults and metacestodes was done according to Detwiler and Criscione (2011) and Detwiler et al. (2017), whereas PCR for oncospheres included some modifications. First, the prior studies used the M13 method to label forward primers, but to increase fluorescence signal in genotyping for oncospheres all forward primers were directly labelled with the same fluorophore (Applied Biosystems: 6FAM). Second, PCR amplifications were performed in 10 μl reaction volumes with the addition of a PCR enhancer (Ralser et al., 2006). Finally, the thermocycler profile was 94°C for 5 min, followed by 39 cycles of 94°C for 30 s, 53°C for 45s, 65°C for 45s, followed by an extension of 65°C for 30 min. Modifications were necessary as we had a lower amplification success for oncospheres (approximately 66%–94% success across families in getting any amplification, see also Detwiler and Criscione 2011). We suspect the lower success was due in part to the much smaller size of oncospheres (~21 × 25 μm, length × width; Criscione and Font 2001b) compared to metacestodes (~184 × 121 μm; Criscione 2000). Fragments were visualized on a 3730*xl* 96-Capillary Genetic Analyzer using a 500 LIZ size standard at the DNA Analysis Facility on Science Hill at Yale University, USA. Binning and scoring of alleles were done manually using Genotyper v.3.7 (Applied Biosystems) by two people.

2.3 | Statistical analyses

Our data set consisted of a total of 17 tapeworms (i.e. maternal families) obtained from six gecko hosts. One to three microsatellite loci were used to unambiguously classify an offspring individual of a maternal tapeworm as the result of a selfing or an outcrossing event. We note the adult parasites exist in a closed mating environment bounded by a gecko host; thus, we know the genotypes of all potential paternal parents. Because of the lower PCR efficiency in oncospheres compared to that of metacestodes, we restricted the study to families that enabled unambiguous classification of offspring (see Method A of Detwiler et al. 2017). Hence, the number of families is not as large as that used in Detwiler et al. (2017). Oncosphere and metacestode genotype data sets for all families are given in Table S1. Within a family, the same genotype calling and error assessment (see below) criteria were applied to both oncospheres and metacestodes. Thus, although the ability to assess error may vary among families due to differences in loci number or quality, there should be no call or error assessment bias between oncospheres and metacestodes within a family.

The relative fitness of selfed offspring, *w*, can be estimated by comparing selfing rates from sequential life history stages; $w = (t_o s_m / s_o t_m)$ (equation 8 in Ritland 1990). Here, s_o and t_o are the selfing and outcrossing rates, respectively, from the oncosphere stage, and s_m and t_m are the rates from the metacestode stage. Ritland's (1990) equation is equivalent to using the odds ratio of trait states across time periods to estimate *w* (Manly, 1985), and therefore, statistical tests of odds ratios can be conducted. In other words, if the odds of being a selfed tapeworm are significantly smaller in the metacestode stage than the odds of being a selfed tapeworm from oncosphere stage (i.e. *w* = odds ratio <1), then ID occurred across infection of the intermediate host.

We tested the null hypothesis that infection through the beetle intermediate host will not differentially impact the survival of selfed relative to outcrossed offspring using the Cochran–Mantel–Haenszel (CMH) test (Cochran, 1954; Mantel & Haenszel, 1959). The CMH analysis tests for an association between life history stage (oncosphere and metacestode) and offspring inbreeding status (selfed and outcrossed) while controlling for categorical stratification (the 17 maternal families). We conducted the CMH test with the continuity correction for the chi-square statistic using the *mantelhaen.test* function in the native *stats* package in R v. 3.4.4 (R Core Team, 2018). The CMH test also estimates a common odds ratio, which in our study would be the common measure of *w* for the selfed offspring. However, this common measure assumes the odds ratio is the same across families. We tested the latter assumption with the Woolf test of homogeneity for odds ratios across strata (tapeworm families) using the *rmeta* package (Lumley, 2018). We measured ID as $\delta = 1 - w$ over the entire data set where *w* was the common odds ratio from the CMH test. We also did a logistic regression where the odds of being a selfed offspring (logit link) were a function of stage (metacestode vs. oncosphere) as a fixed factor. Random effects included gecko host of origin, family nested in gecko host and an interaction between family nested in gecko host and stage. The test of the interaction is analogous to the Woolf test. Logistic regression models were carried out in R v. 3.5.3 (R Core Team, 2019) in package *lme4* (Bates, Maechler, Bolker, & Walker, 2015) using the generalized mixed model function *glmer*.

To be explicit, we are estimating a stage-specific measure of ID: from developed oncosphere to metacestode. Unfortunately, at this time, we do not have a means to test whether selfed zygotes are less likely to develop to oncospheres in the first place (e.g. in plants, mating systems can be manipulated and then seed set could be compared to assess development). Nonetheless, our analysis occurs across a critical life history stage of the parasite where host–parasite compatibilities could come into play, and hence, ID could be manifested.

2.4 | Error rate estimation and adjustment

The above analyses were conducted on the "original" data set, where we removed offspring at either stage with evidence of allele dropout (see example in Table S1). Although we omitted the offspring with dropout genotypes from the original data set, we did use these individuals to estimate an error rate on the identification of outcrossed individuals.

Assuming that these dropout genotypes were the result of a true outcross, the error rate for each stage within each maternal family was calculated as follows. First, the total "true" number of outcrosses (O_T) was the sum of the dropout genotypes (O_D) and the number of identified outcrossed offspring (O_I) in the original data set. The probability of nondetection of a true outcross is then $\varepsilon = O_D/O_T$. This error rate acts as a lower bound of dropout rates as not all dropouts are possible to detect. Nonetheless, as the same criteria were applied to both stages within a family, it is possible to compare relative differences in dropout error for the two stages and subsequently apply an error correction to determine how dropout influences the results. Next, we adjusted the original data set to account for the error rate. First, the error rates were averaged across families for the metacestodes ($\bar{\varepsilon}_m$) and oncospheres ($\bar{\varepsilon}_o$). Then for each family and stage within, we adjusted the number of outcrossed individuals in the original data set according to the formula $O_A = O_I/(1-\bar{\varepsilon}_i)$ where the denominator represents the probability of detecting an outcross for stage *i*. O_A values were rounded to the nearest integer, and the total number of offspring was kept constant such that the number of selfed offspring was reduced by the same increase from O_I to O_A. For example, in Host 7 Worm B, there were 81 oncospheres genotyped and O_I = 42. Overall, $\bar{\varepsilon}_o$ = 0.054 (see Section 2.1), so O_A = 44 and the number of selfed oncospheres was reduced from 39 to 37 in the error-adjusted data set (Table S2). Using the error-adjusted data set, we repeated the statistical tests on the odds ratios for each family and the common odds ratio from the CMH test.

3 | RESULTS

In the original data set, a total of 2,931 offspring (1,598 metacestodes and 1,333 oncospheres) were genotyped across the 17 maternal families. There was an average of 94 metacestodes per family (range: 92–95), whereas in the oncospheres, the average was 78.4 (range: 51–92; Table S2).

For the original data set, odds ratios among the families (i.e. *w* of selfed offspring across infection of the intermediate hosts) ranged from 0.48 to 1.62 (Table S2; Figure 1a). Four families had odds ratio greater than one, whereas the rest were less than one (Figure 1a). However, only in two families was there significant ID such that the odds ratios were significantly less than one (Host 7 Worm A, w = 0.48, 2-sided p = 0.023; Host 7 Worm C, w = 0.55, 2-sided p = 0.047). The CMH test was significant (X^2 = 7.19, df = 1, p = 0.007) returning a common odds ratio (w) of 0.81 (95% CI: 0.69–0.94, Figure 1a). The Woolf test was not significant (X^2 = 18.85, df = 16, p = 0.28) indicating homogeneity of odds ratios among families. Hence, the original

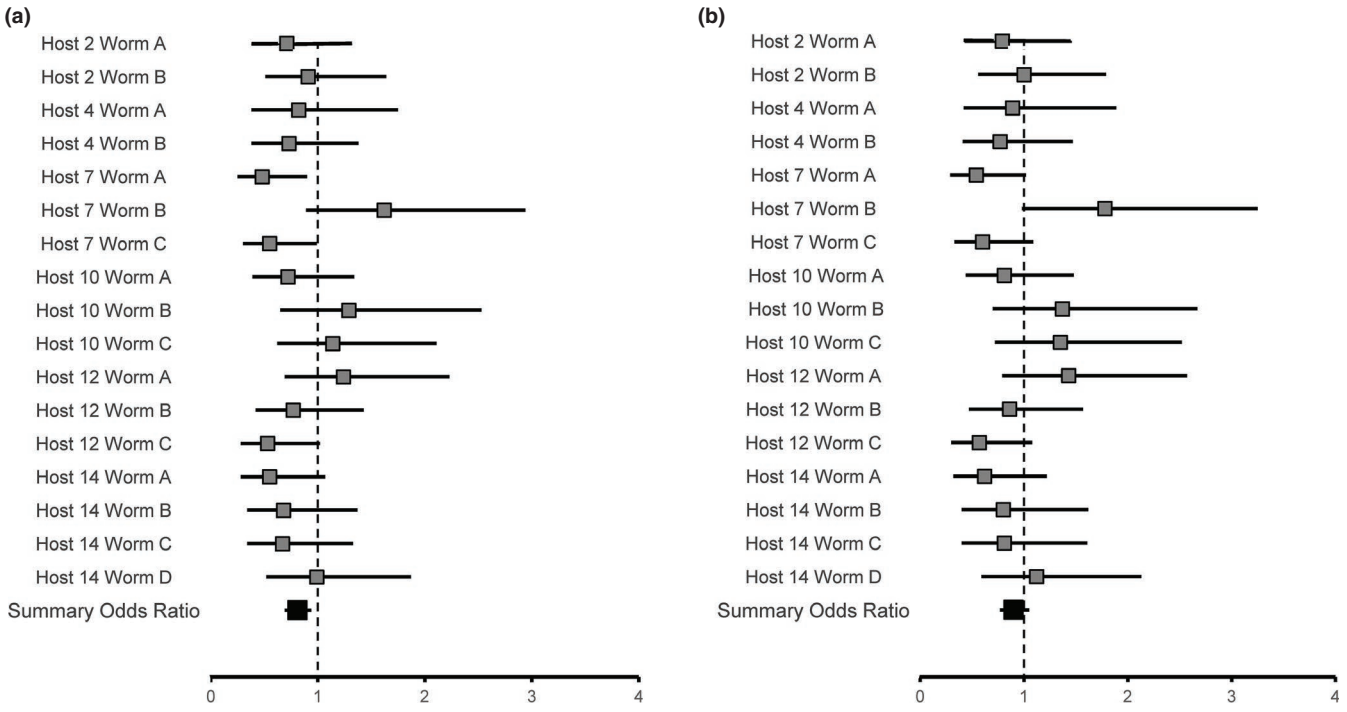

FIGURE 1 Forest plots with family odds ratios (w = relative fitness of selfed to outcrossed offspring) and summary odds ratio from the CMH test. 95% CI is shown for each family level estimate (grey boxes) and the summary odds ratio (black box). The summary odds ratio in both graphs (a) and (b) is shown at the bottom. The dotted vertical line represents where the odds ratio = 1, which indicates fitness selfed = fitness outcrossed (a) Original data set. (b) Error-adjusted data set (see text for explanation)

data set indicates significant, but low ID with a common level of $\delta = 0.19$ (95% CI: 0.06–0.31).

The original data set excluded offspring with dropout genotypes. We observed 38 individuals across seven families with dropout genotypes among the oncospheres and three dropout genotypes across three families among the metacestodes (Tables S1 and S2). The average probabilities of detecting an outcrossed offspring were 0.946 ($\bar{\epsilon}_o = 0.054$) and 0.995 ($\bar{\epsilon}_m = 0.005$) at the oncosphere and metacestode stages, respectively. Therefore, the odds of incorrectly classifying an outcrossed offspring as a selfed offspring were over 12 times higher among the oncospheres. This is an expected result because there was lower amplification success for oncospheres.

After applying the error estimates to both stages, no families had a significant deviation from an odds ratio of 1 (Table S2; Figure 1b). In the adjusted data set, the odds ratios among the families ranged from 0.54 to 1.78 with six families having an odds ratio at or greater than one (Figure 1b). The CMH test was no longer significant ($X^2 = 1.63$, $df = 1$, $p = 0.201$) and the Woolf test remained nonsignificant ($X^2 = 19.17$, $df = 16$, $p = 0.26$). The common odds ratio was 0.9 (95% CI: 0.77–1.05; Figure 1b) leading to a nonsignificant estimate of ID, $\delta = 0.1$ (95% CI: −0.05 to 0.23). Hence, the error-adjusted results show that the greater potential for dropouts in the oncospheres biases the selfing rates higher at this life history stage. Consequently, levels of ID would be overestimated.

The $\bar{\epsilon}_0 = 0.054$ in the oncospheres led to an average of two selfed offspring per family changed to an outcross status, whereas there were no changed values among metacestodes due to their low error. For heuristic purposes, we asked at what level of error an adjusted data set would no longer show significance in the CMH test. A value of $\bar{\epsilon}_0 = 0.029$ was enough to drive the CMH test nonsignificant ($w = 0.86$, CI: 0.74 to 1, $X^2 = 3.67$, $df = 1$, $p = 0.055$). This level of error amounts to changing on average just one selfed oncosphere per family to an outcross status.

After model comparisons, the logistic regression models yielded nearly identical results both in terms of no significant heterogeneity of odds ratios among families (i.e. there was no interaction with stage and family nested within gecko host) and in point estimates of the common odds ratio (difference in the thousandths place compared to the CMH estimates). Inclusion of gecko host in the model had virtually no impact on the point estimate of the odds ratio, and model comparisons indicated the more simple model of stage (fixed effect) and family as the sole random effect was preferred (see Table S3 for all model comparisons and point estimates in the original and error corrected data sets).

4 | DISCUSSION

For the tapeworm *O. javaensis*, the data indicate little to no ID across infection of an intermediate host. This result has relevance to the primary mating system of *O. javaensis* itself and to the broader context of understanding the interplay between hermaphroditic mating systems and ID. For the former, the little to no ID means that the mixed-mating system identified by Detwiler et al. (2017) reflects the primary mating system of *O. javaensis*. Specifically, the population level selfing rates estimated by Detwiler et al. (2017) ranged from 19.4% to 52.2% among five locations and had a mean of 30.6%. These estimates were made with metacestode progeny-array data and were a function of the infection intensities among hosts in each location as well as assuming random reproductive success among individuals (Detwiler et al., 2017). In general, the primary mating system would be better reflected by using oncospheres because they are the first measurable developmental stage for *O. javaensis*. Obviously, if there is no ID across infection of the intermediate host, then the selfing rate estimates given in Detwiler et al. (2017) would be the same even if progeny-array data were based on the oncospheres. With the error-adjusted data set, we could not reject the hypothesis of no ID. Even if we take the original data set at face value and, thus, assume low but significant ID, the estimate of the primary selfing rate based on the oncosphere stage would only be 35.2%. This estimate is based on the equation given in Maki (1993) that enables estimation of the primary mating system given a current life history stage selfing rate (30.6% based on metacestodes in our system) and a measure of ID occurring prior to this stage ($\delta = 0.19$ estimated herein). Detwiler et al. (2017) also highlighted how the selfing rate of *O. javaensis* could be elevated due to density-dependent fecundity (a.k.a. crowding) such that tapeworms from lower intensity infections, which have higher individual selfing rates, contribute more offspring to the pool of infective propagules. Assuming crowding, population selfing rate estimates ranged from 47.3% to 67.1% among five locations and had a mean of 58.9% (figure 2 in Detwiler et al. (2017)). Using the correction for possible ID, the primary selfing rate based on oncospheres would be 63.9%. So, regardless if we take the average actual mating behaviour of individuals based on random reproductive success or the manifestation of the selfing rate in the pool of infective propagules due to crowding as the primary mating system, *O. javaensis* remains within the mixed-mating category even after accounting for possible ID.

How does the mixed-mating system and no to little ID of *O. javaensis* compare to the results of the meta-studies? Using a dichotomous classification among plants, Husband and Schemske (1996) found that predominantly selfing populations ($s > 0.55$) had mean $\delta = 0.23$, whereas outcrossers ($s < 0.45$) had mean $\delta = 0.53$. With an updated data set and larger sample sizes, Winn et al. (2011) revisited the topic in plants but using the three-category classification of mating systems (see Section 1). Similar to the results of Husband and Schemske (1996), they found a mean $\delta = 0.48$ for outcrossers and mean $\delta = 0.23$ for selfers. Of primary interest to Winn et al. (2011) was to test whether mixed-mating taxa would have intermediate levels of ID as would be expected if mixed mating represented an evolutionary transition between largely selfing and largely outcrossing. Interestingly, mixed-mating taxa had a mean $\delta = 0.51$ and this value did not significantly differ from outcrossers (see figures 3 and 6 in Winn et al. 2011). They discuss that purging in some mixed-mating taxa was likely precluded by selective interference. Their arguments

were supported in part by a subset of their data where adult-estimated inbreeding coefficients were close to 0 in the outcrossing and mixed-mating groups (0.006 and 0.038, respectively), whereas the selfers had a value of 0.545. So even if a species has a primary mixed-mating system, the 0 inbreeding coefficient estimated in adults indicated selfed individuals were not surviving to adulthood (i.e. there was selective interference).

Comparison to the animal-based study is not as straightforward due to the fact that apparent ID (apparent-δ) is measured; however, Escobar et al. (2011) provide a measure of max ID (max-δ), which is based on the assumption of 100% survival of outcrossed offspring. Therefore, true δ lies in between apparent-δ and max-δ (Escobar et al., 2011). Using the data points given in figure 4a (actual numbers obtained from their tables 2 and 3) of Escobar et al. (2011), the mean apparent-δ = −0.055 and mean max-δ = 0.208 for selfers (n = 6 for both), whereas in outcrossers, the mean values were 0.355 (n = 6) and 0.616 (n = 5), respectively. The mixed-mating taxa had a mean apparent-δ = 0.436 (n = 5) and mean max-δ = 0.727 (n = 3). Qualitatively, the levels of ID in the three mating system categories are similar between the animal (Escobar et al., 2011) and plant data sets (Winn et al., 2011).

As noted in the Introduction, there is a high variance in δ among the mixed-mating taxa reported in the meta-analyses (Escobar et al., 2011; Winn et al., 2011). Clearly, the greater allele-dropout error in oncospheres upwardly biases δ in *O. javaensis*. Nonetheless, even if we ignore this error, the estimated value of δ = 0.19 is still in line with the low values reported for both plant and animal selfing taxa. Winn et al. (2011) provide support for selective interference to explain high δ in some mixed-mating taxa. They explicitly state that if mixed mating was an evolutionary transition, then mean ID of mixed-mating taxa should be between the mean of selfers and that of outcrossers. They also mentioned how some models (e.g. pollen discounting; Holsinger, 1991) predicted stable mixed mating with low to moderate ID (Winn et al., 2011, and references therein). Nonetheless, selfing alone may not fully account for all inbreeding in a hermaphroditic population. We argue that another aspect of the mating system, in particular kin mating, has been overlooked and may also explain lower values of ID found among mixed-mating taxa.

The full context of the mating system of *O. javaensis* provides additional insight that may explain low δ among mixed-mating taxa and possibly outcrossing taxa as well. In Detwiler and Criscione (2017), pedigree reconstruction analyses of adult tapeworms among gecko hosts enabled the estimation of realized selfing rates (i.e. proportion of adult individuals that were the product of a selfing event) and the proportion of kin-dyads (full and half sibs) within hosts. There was a highly significant signature that sibling parasites were cotransmitted at a much higher frequency than expected by chance alone. Using the proportions of kin-dyads within hosts, the estimated potential kin-mating rates had a mean of 21.5% among five populations (table 2 in Detwiler and Criscione (2017)). Interestingly, the realized selfing rates (mean of 54.8% among five populations; table 2 in Detwiler and Criscione (2017)) alone could not explain total levels of inbreeding (as estimated with F_{IS}), which had a mean of 0.581 among five populations (table 1 in Detwiler and Criscione (2017)). However, when including both kin-mating and selfing rates into a general inbreeding equilibrium equation (Hedrick & Cockerham, 1986), the observed F_{IS} values could be fully accounted for in each of their five studied populations.

Theoretical work shows that kin mating can be more efficient than selfing in the purging of ID (Porcher & Lande, 2016). In addition, the use of biparental inbreeding to reduce ID has been demonstrated in the management of captive dioecious species (Templeton & Read, 1984). In *O. javaensis*, biparental inbreeding makes a significant contribution to the overall inbreeding in populations (Detwiler & Criscione, 2017). Hence, the lack of or very low ID in *O. javaensis* could potentially be the product of purging facilitated by both selfing and kin mating. A significant issue this result bears on is that the primary selfing rate alone may not be sufficient to classify the mating history of all hermaphroditic species when looking for a relationship to ID. Indeed, Latta and Ritland (1994) acknowledged that primary selfing rates "may not be indicative of historical levels of inbreeding," which as they underscored "is the hypothesized cause of purging."

Interestingly, the mean F_{IS} = 0.581 among the five mixed mating populations of *O. javaensis* was of near equivalence to the mean F = 0.545 reported among selfing taxa in Winn et al. (2011). Similarly, Voillemot and Pannell (2017) reported a F_{IS} = 0.36 and a lack of ID in a self-compatible population of the plant *Linaria cavanillesii*. Highlighting the importance of F, Winn et al. (2011) recommended that studies report inbreeding coefficients to look for evidence of selective interference (i.e. F close to 0 in adults). We support this advice, but for another reason: an inbreeding coefficient greater than predicted from a primary selfing rate under inbreeding equilibrium (see Section 1) may be indicative of kin mating significantly driving the overall inbreeding in the population. Thus, even if primary selfing rates are low, high levels of inbreeding, and hence the potential for purging, could still be driven by kin mating. We fully acknowledge Escobar et al.'s (2011) sentiment that technical or biological factors could drive up F_{IS} estimates. However, in connection with other population genetic statistics or patterns, the robustness of F_{IS} estimates could be examined (Waples, 2015, 2018). For example, a higher selfing rate estimated from adults using identity disequilibrium (David, Pujol, Viard, Castella, & Goudet, 2007; Jarne & David, 2008) compared to an early-stage, progeny-array-based selfing rate could indicate a role for kin mating as biparental inbreeding also increases identity disequilibrium.

Assessing the relationship between mating system and ID among parasitic flatworms is limited as there are few systems for which both the mating system in nature has been characterized and a measure of δ has been estimated from the same locations. Here, the mixed-mating tapeworm *O. javaensis* was found to have little to no ID from the oncosphere to metacestode stage. It may be possible that different genetic compatibilities (e.g. Zhong, Pai, Wang, Keech, & Yan, 2013) between this parasite and its "natural" (unknown) intermediate host(s) could alter the outcome. However, it is not possible with the current data to say whether ID would increase or decrease relative to the intermediate host we used.

Nonetheless, we also note that in Detwiler and Criscione (2017), the estimated population selfing rates based on the crowding assumption of the metacestode progeny-array data were not statistically different than the realized selfing rates based on the adult pedigree reconstruction data. Hence, there was no evidence of ID occurring from the metacestode to adult stage either. Focusing on the selfing rate alone, *O. javaensis*, would be classified as a mixed-mating species with low to no ID across much of its lifespan. But, in the context of its high F_{IS} (due to both selfing and kin mating; Detwiler & Criscione, 2017), *O. javaensis* supports the hypothesis that highly inbred species should be purged of their ID. Studies on the trematode *Coitocaecum parvum* are also consistent with purging in an inbred species. Lagrue and Poulin (2009) did not find any differences in several infectivity/fitness traits between forced-selfed and permitted-outcrossing treatments. The primary selfing rate of this parasite is not known, but it has a life history trait, sexual maturation during its encysted stage, that forces self-mating. Indeed, *Coitocaecum parvum* has high levels of inbreeding with F_{IS} ranging from 0.73 to 0.99 among 12 microsatellite loci (Lagrue & Poulin, 2009); we note the latter range excludes potentially duplicated loci in their data set (Detwiler & Criscione, 2011).

Studies on the tapeworm *Schistocephalus solidus* support the opposite end of the spectrum where outcrossing species are expected to show high ID. Various traits such as hatching rates and infection success (quantified as per cent, numbers and larval size) of first and second intermediate hosts were higher in outcross treatments compared to forced-selfed treatments (Christen, Kurtz, & Milinski, 2002; Christen & Milinski, 2003; Milinski, 2006; Schjorring & Jager, 2007). From these studies, Escobar et al. (2011) reported a mean apparent apparent-δ = 0.78 and max-δ = 0.93, though it is not known whether averaging was done across all traits. An eloquent study by Benesh et al. (2014) reported a lifetime apparent-δ = 0.9 for *S. solidus*. Interestingly, their experiments showed purging where ID was lower after a second generation of selfing. Benesh et al. (2014) indicated *S. solidus* is not inbred in nature (stating F_{IS} = 0 from unpublished data) and thus suggesting that selective inference may preclude purging in natural populations of *S. solidus*. The trematode *Diplostomum pseudospathaceum* is also a predominately outcrossing species (F_{IS} = 0 across five locations in Finland; Louhi, Karvonen, Rellstab, and Jokela (2010)). However, a study using samples from a population in Germany was not conclusive for ID (Rieger, Haase, Reusch, & Kalbe, 2013). Experiments using individuals of single clones (selfing treatment) vs mixed clone infections (outcrossing treatment) were consistent with ID in that there was lower hatching and infection success across first and second intermediate hosts for selfed vs outcrossed treatments (see figures 1–3 in Rieger et al. (2013)). They reported, though, that after accounting for clonal lines in their statistical models, there was no support for ID (Rieger et al., 2013).

To conclude, our approach to estimating δ (comparison of selfing rates from different life history stages; Ritland (1990)) is different than the typical apparent-δ found in animal-based studies. In addition to Detwiler and Criscione (2017), Jokela, Wiehn, and Kopp (2006) appears to be the only other animal-based study that has used this approach. Although the method may have the disadvantage of not being able to tease apart the role of ID on particular life history traits (e.g. body size), not all species have the same set of shared traits to compare. For example, egg hatching rates have been used to assess ID in parasite systems (e.g. Benesh et al., 2014; Christen et al., 2002; Lagrue & Poulin, 2009). For *O. javaensis*, there is no egg hatching stage as oncospheres are directly eaten by an intermediate host. Also, it may be difficult to design experiments without knowing how many selfed individuals are present in the first sampled stage, though some preliminary data would help determine whether the comparison of selfing rates from different life history stages is a suitable approach. Nonetheless, comparison of selfing rates does offer several benefits that should facilitate additional ID studies among parasitic flatworms. First, it is a direct measure of δ (in contrast to the underestimated apparent-δ) that assesses the relative survivorship of selfed versus outcrossed individuals between two life history stages. Hence, it takes into account the cumulative effects of various traits that might be affected by ID. Second, Christen et al. (2002) and Christen and Milinski (2003) noted differences between selfed and outcross treatments may only be manifested under competitive situations. The comparison of selfing rates from different life stage samples necessarily includes possible competing interactions between selfed and outcrossed individuals. Third, as discussed in Detwiler and Criscione (2017), field-based estimates of δ could be estimated by using pedigree reconstruction-based selfing rates obtained from different life history stages.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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