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Role of parasite transmission in promoting inbreeding: I. Infection intensities drive individual parasite selfing rates

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Abstract

Among parasitic organisms, inbreeding has been implicated as a potential driver of host–parasite co-evolution, drug-resistance evolution and parasite diversification. Yet, fundamental topics about how parasite life histories impact inbreeding remain to be addressed. In particular, there are no direct selfing-rate estimates for hermaphroditic parasites in nature. Our objectives were to elucidate the mating system of a parasitic flatworm in nature and to understand how aspects of parasite transmission could influence the selfing rates of individual parasites. If there is random mating within hosts, the selfing rates of individual parasites would be an inverse power function of their infection intensities. We tested whether selfing rates deviated from within-host random mating expectations with the tapeworm *Oochoristica javaensis*. In doing so, we generated, for the first time in nature, individual selfing-rate estimates of a hermaphroditic flatworm parasite. There was a mixed-mating system where tapeworms self-mated more than expected with random mating. Nevertheless, individual selfing rates still had a significant inverse power relationship to infection intensities. The significance of this finding is that the distribution of parasite infection intensities among hosts, an emergent property of the transmission process, can be a key driver in shaping the primary mating system, and hence the level of inbreeding in the parasite population. Moreover, we demonstrated how potential population selfing rates can be estimated using the predicted relationship of individual selfing rates to intensities and showed how the distribution of parasites among hosts can indirectly influence the primary mating system when there is density-dependent fecundity.

KEYWORDS
hermaphrodite, inbreeding, infection intensity, mating system, parasite, selfing rate

1 | INTRODUCTION

Inbreeding has been implicated as a driver of host–parasite co-evolution, drug-resistance evolution and parasite diversification (Agrawal & Lively, 2001; Price, 1977; Schwab, Churcher, Schwab, Basanez, & Prichard, 2006). In general, mating systems that lead to inbreeding can influence the evolution of a species by magnifying the effect of drift, reducing the effective recombination rate and altering selection efficiency (Charlesworth, 2003; Hartfield, 2016; Nordborg, 2000; Pollak, 1987). Despite the potential influence of inbreeding on parasite evolution/co-evolution, there are still key gaps in our knowledge of what parasite life history traits influence inbreeding in natural populations. For example, from hermaphroditic mating system studies predominantly conducted on plants and molluscs (Goodwillie, Kalisz, & Eckert, 2005; Jarne & Auld, 2006), the primary mating system (self-mating vs. outcrossing) is known to be a major factor that shapes inbreeding within populations (Charlesworth, 2003). Yet, there are no direct estimates of individual selfing rates for any parasitic flatworm in nature.

Neodermata Platyhelminthes (flukes, tapeworms and monogenes) are a major parasitic group with over 130,000 estimated species (Strona & Fattorini, 2014), most of which are hermaphroditic. Nonetheless, mating system dynamics largely remain a black box for these ecologically diverse and, in some cases, medically or economically important organisms (Roberts, Janovy, & Nadler, 2009). To date, Jarne and Auld (2006) provide the only quantitative synthesis of parasitic flatworm mating systems. In their review, the mating systems of two parasitic species were directly assessed through progeny-array data and 12 parasitic species were indirectly assessed via population estimates of Wright's F_{IS}, which quantifies deviations from heterozygosity expected under Hardy–Weinberg equilibrium. As noted by Jarne and Auld (2006), there are caveats to using F_{IS} to estimate the primary mating system. Assuming a constant rate of self-mating over generations (i.e., inbreeding equilibrium), F_{IS} is related to the selfing rate (s) as $F_{IS} = s/(2 - s)$ (Hedrick, 2011). However, s estimated from F_{IS} can be artificially increased due to technical factors (e.g., null alleles), the Wahlund effect or kin mating (Jarne & David, 2008). Selection can also affect an estimate of s from F_{IS}. If inbred offspring do not survive to adulthood, that is, there is inbreeding depression, F_{IS} estimated from adults and hence s would be reduced relative to that expected from the primary mating system (Ritland, 1990). Thus, the best way to assess the mating system of individuals is via direct assessment of parentage (progeny-array data) (Jarne & David, 2008).

Admittedly, progeny-array data are difficult to generate in parasitic organisms. Historically, there are some impressive studies that used radiolabelled sperm to show cross-insemination (Nollen, 1983). Unfortunately, this approach does not verify fertilization, and thus, selfing rates cannot be obtained. Currently, there are only four parasitic flatworm species where progeny-array data have been generated to assess selfing/outcrossing (Lüscher & Milinski, 2003; Rieger, Haase, Reusch, & Kalbe, 2013; Schelkle, Faria, Johnson, van Oosterhout, & Cable, 2012; Trouvé, Renaud, Durand, & Jourdane, 1996, 1999). All of these studies are laboratory-based experiments. In natural populations, therefore, we still do not know how pervasive self-mating is among parasitic flatworms much less if there are ecological factors or parasite life cycle attributes that can predispose parasitic flatworms to inbreeding.

Our goal was not only to estimate the mating system of individual parasitic flatworms in nature but also to elucidate basic ecological features of parasite life history that could impact whether an individual self-mates or outcrosses. In particular, we addressed how the distribution of parasites among hosts, an emergent property of the transmission process, could impact the primary mating system. As a framework, we first recognize that for most metazoan endoparasites, adult breeders are separated among individual hosts (i.e., there is a closed mating system such that individuals cannot mate with parasites in another host; Criscione & Blouin, 2005). If there is random mating within hosts, the selfing rates of individual parasites would be the inverse of the infection intensity (number of parasite individuals in a host) experienced by those parasites (dashed curve in Figure 1) and the population-level selfing rate (i.e., the average selfing rate across all parasites from all hosts) would simply be the inverse of the mean infection intensity (see Materials and Methods for a proof of the latter).

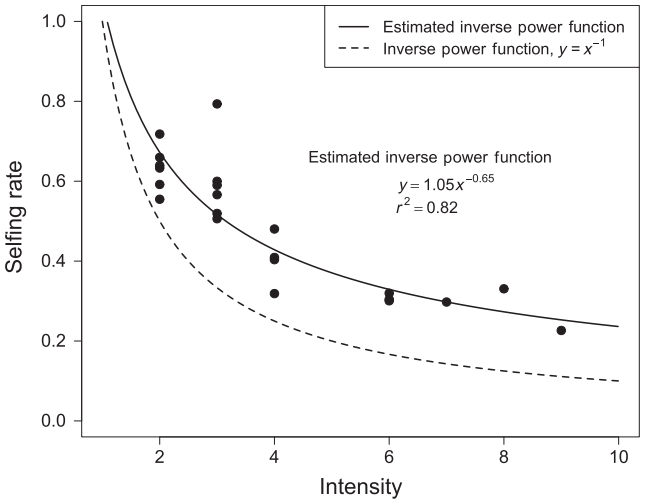

FIGURE 1 Inverse power relationship between parasite selfing rate and infection intensity based on the GLM. The dashed line shows the null expectation of the selfing rate based on within-host random mating. The solid line shows the GLM estimated inverse power function where each dot is an average within-host parasite selfing rate

Our study provides the first nature-derived, direct estimates of the primary mating system of a hermaphroditic flatworm parasite, the tapeworm *Oochoristica javaensis*. We tested whether selfing rates deviated from within-host random mating expectations and, in doing so, tested whether parasite inbreeding as manifested by self-mating is dictated by the distribution of parasites among hosts, a fundamental component of parasite transmission. We then demonstrated how the distribution of parasites among hosts can be used to generate a potential population-level estimate of the selfing rate. We also highlighted how the distribution of parasites among hosts can indirectly influence population-level selfing rates when there is density-dependent fecundity (i.e., crowding effects; Dobson, 1986; Read, 1951). The methods we employed will aid future experimental- or field-based mating system studies of parasitic flatworms and animal hermaphrodites in general.

2 | MATERIALS AND METHODS

2.1 | Study system

The hermaphroditic tapeworm *O. javaensis* has a terrestrial life cycle involving two hosts. Various gecko species, particularly the Mediterranean gecko *Hemidactylus turcicus* in the southern United States, can serve as the definitive host, the host where the parasite sexually matures (Criscione & Font, 2001a, 2001b, 2001c; Kennedy, Killick, & Beverley-Burton, 1982). The body (strobila) of *O. javaensis* consists of a series of repeated segments (proglottids). Anterior proglottids are immature (i.e., reproductive organs are not yet developed). Going

towards the posterior, proglottids begin to mature and then begin to show evidence of germination, and finally, the most posterior proglottids are gravid (i.e., they contain the diploid larvae called oncospheres). Posterior proglottids ultimately detach whole and are passed in the faeces of the reptile host. Oncospheres are then consumed 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). The larval cestodes develop into an infective juvenile stage (generically termed a metacestode; Chervy, 2002; Conn, 1985) within the coelom of the beetle, and the life cycle perpetuates when infected beetles are consumed by the gecko.

2.2 | Field collections

From May to October 2011, Mediterranean geckos were collected by hand from dusk to midnight from buildings in five locations within College Station, Texas, USA. These locations are described in detail, including a map, in Detwiler and Criscione (2014). The Mediterranean gecko is an invasive species (nonendangered and not protected) in the United States and does not require collecting permits in the state of Texas. The research protocols (i.e., capture, handling and sacrifice [decapitation followed by pithing] prior to dissection) in this study were approved by the Institutional Animal Care and Use Committee at Texas A&M University (AUP 2009-23 and 2012–023). During dissections, live tapeworms were collected from the gecko's intestine.

Live tapeworms were examined under a stereomicroscope to determine the developmental stage of the tapeworm (immature, mature or gravid). Tapeworms were deemed immature if no mature segments were observed. Mature individuals had segments with fully developed ovaries and testes, but no gravid segments (i.e., there were no proglottids with developed oncospheres). Gravid tapeworms had mature and gravid segments. If gravid segments were observed, fine forceps were used to separate eight gravid segments from the rest of the tapeworm. The remaining part of each tapeworm was heat-killed in 90°C water (Criscione & Font, 2001a) and preserved in 70% ethanol for subsequent microsatellite genotyping and morphological work.

The isolated, live gravid proglottids were placed onto a piece of filter paper in a small plastic petri dish (35 mm × 10 mm). A small amount of flour was dusted onto the tapeworm tissue, and then, 10 starved beetles (for 24 hrs) were placed into each dish (Criscione & Font, 2001a). After 20 days, the beetles were dissected and all the tapeworm metacestodes were counted and preserved in 70% ethanol for genotyping. Although it would have been preferable to genotype oncospheres to obtain offspring genotypes, we could not consistently amplify microsatellite loci from individual oncospheres (Detwiler & Criscione, 2011). Metacestodes were used because they provided ample DNA for PCR. We note that because our estimates of the primary mating system came from the metacestode stage, we are assuming there was no appreciable amount of selection against selfed parasites within the beetle host. Even if there was inbreeding depression from the oncosphere to metacestode stage, it is hard to envision how this could generate a relationship between the within-gecko infection intensity and selfing rate when analysed at the metacestode stage. Also, a similar level of inbreeding depression among inbred offspring should not preclude detection of the relationship at the metacestode stage. However, variable inbreeding depression among inbred offspring could create more noise in the data reducing the chances to detect a relationship at the metacestode stage.

2.3 | Adult and larval parasite genotyping

DNA extraction and microsatellite genotyping followed that of Detwiler and Criscione (2011). Parent tapeworms were genotyped using the scolex and neck region, which does not contain any maturing proglottids. Parent tapeworms from each host were genotyped with 26 microsatellite markers (di005, di019, di035, di068, di097, di109, tri007, di008, di011, di033, di046, di073, di131, di140, tet007, tri022, di001, di030, di044, di069, di078, di086, di094, di032, tet012 and tri001; Detwiler & Criscione, 2011) to determine loci that could be used to estimate selfing rates of individual worms. Next, loci to genotype offspring (metacestodes) of a specific parent were selected based on the criterion that the locus (or combination of loci) could be used to estimate the selfing rate of that parent tapeworm (described below). The number of loci genotyped in the offspring of a parent worm ranged from 1 to 6 (discussed below). Based on simulations (Fig. S1) and the technical feasibility of extracting metacestodes in a 96-well format, we aimed to genotype 95 metacestodes from a given parent. The metacestodes used for genotyping were randomly selected from the infected beetles. All adult and offspring genotypes were visualized on a 3730xl 96-Capillary Genetic Analyzer with 500 LIZ size standard at the DNA Analysis Facility on Science Hill at Yale University, USA. Alleles were scored and manually inspected with GENOTYPER 3.7 (Applied Biosystems). All adult genotypes were scored by JTD and then independently checked at random by ICC.

2.4 | Estimating the individual selfing rates of parent worms

Because the tapeworms exist in a closed mating system, we could determine the genotypes of all potential parents of a given progeny. Moreover, we knew the maternal parent because gravid proglottids were obtained directly from the maternal tapeworm. Ideally, complete parentage would be the best means to estimate individual worm selfing rates. However, because polymorphism at microsatellite loci was low in populations of *O. javaensis* (Detwiler & Criscione, 2017), some parents shared the same alleles across loci making complete parentage impossible. Thus, depending on the polymorphism within and among adult tapeworms in a host, various methods were used to estimate individual tapeworm selfing rates. Table S1 provides the raw genotype data and method of analysis (Methods A–D described below) used to generate each individual tapeworm selfing-rate estimate.

2.4.1 | Method A

In over half the cases, a single locus or a combination of loci allowed unambiguous determination of whether the progeny of a maternal worm was the result of a selfing event or outcrossing event. For example, in Host 2, maternal Worm A had genotype 237/237 and Worm B had genotype 245/245 at locus di030 (Table S1). In this example, the estimated selfing rate ($\hat{s}$) of Worm A is the proportion of progeny that had a 237/237 genotype. The variance using Method A is the variance of the binomial distribution.

2.4.2 | Method B

Within some hosts, a maternal tapeworm was homozygous at one or more loci whereas one or more other potential paternal parents were heterozygous with one allele also present in the maternal tapeworm. For example, in Host 1, maternal Worm A was 113/113 and Worm B was 113/117 at locus di033. Two other independent loci (i.e., recombination rate is 0.5; discussed at the end of Method C) showed the same pattern (Table S1). In this case, a 113/117 genotype in the progeny of Worm A is a discernable outcrossed progeny because of the presence of a nonmaternal allele. Even though a 113/113 genotype is ambiguous, as it can result from a selfing or outcrossing event, $\hat{s}$ can still be estimated using the proportion of discernable outcrossed progeny (n_t/N, where n_t is the number of discernable outcrossed progeny and N is the total number of progeny genotyped from a given maternal tapeworm).

Estimating $\hat{s}$ with Method B is a special case of the methods presented in Cruzan, Hamrick, Arnold, and Bennett (1994) and Shaw, Kahler, and Allard (1981). The proportion of discernable outcrossed progeny, n_t/N, is the product of the outcrossing rate ($t = 1 - s$) times the probability of detecting a discernable outcrossed progeny ($1 - \alpha$, where α is the probability of nonidentification of an outcross). In Shaw et al. (1981), who were estimating population-level selfing rates, and Cruzan et al. (1994), who were estimating individual plant selfing rates in an open population, α is a random variable (estimated from the frequencies of maternal alleles in the paternal pool) that has an associated variance. Because adult endoparasites have closed mating systems, we can identify genotypes of all potential parents. Thus, α can be determined without error from Mendelian expectations. Henceforth, we designate it as α_m. In a cross with maternal genotype 11 × paternal 12, α_m in the progeny of the maternal individual is .5. If there are x independent loci that show the same pattern, then the multilocus $\alpha_m = .5^x$. The probability of detecting a discernable outcrossed progeny increases rapidly with more loci (e.g., with 4 loci, $1 - \alpha_m = .9375$). Using the above information, the following equation can be used to estimate $\hat{t}$ of an individual tapeworm:

$$\hat{t} = \frac{n_t}{N(1 - \alpha_m)}. \quad (1)$$

Subsequently, $\hat{s}$ is estimated from $1 - \hat{t}$. The variance of $\hat{t}$ (see Shaw et al., 1981) is equal to the variance of $\hat{s}$ and is given by

$$\mathrm{Var}\,\hat{t} = \frac{\hat{t}[1 - \hat{t}(1 - \alpha_m)]}{N(1 - \alpha_m)}. \quad (2)$$

Equation 1 can be used to estimate $\hat{t}$ of the maternal tapeworm when there is more than one potential paternal tapeworm as long as a potential paternal tapeworm is not homozygous for the same allele as the maternal tapeworm, for example, maternal genotype of 11 and two potential paternal individuals with genotype 12. In this latter example, $\hat{t}$ is the total outcrossing rate, but does not distinguish the proportion of outcrossing between the two potential paternal individuals. Nonetheless, $\hat{s}$ of the maternal parent, the statistic of interest in this study, can still be estimated as $1 - \hat{t}$.

2.4.3 | Method C

The method of Cruzan et al. (1994) was designed for open populations and relied upon a nonmaternal allele being present in the progeny to estimate $\hat{t}$. Thus, if a locus is biallelic and the maternal individual is a heterozygote, an estimate of $\hat{t}$ is not possible (Ritland, 2002). However, in closed mating systems, Mendelian expectations enable the estimation of $\hat{s}$ from the observed proportion of discernable selfed genotypes, n_s/N. For example, in Host 8, maternal Worm C had genotype 120/122 and potential paternal Worms A and B had genotype 120/120 at locus di001. There were three other independent loci with this same pattern (Table S1). In fact, because Worms A and B had the same multilocus genotype across 26 loci, we could not obtain individual selfing estimates for either of these tapeworms. However, in offspring of Worm C, the genotype 122/122 is a discernable selfed genotype whereas genotypes 120/120 and 120/122 are ambiguous. Here, n_s/N is the product of s times the probability of detecting a discernable selfed progeny ($1 - \beta_m$, where β_m is the probability of nonidentification of a selfing event according to Mendelian expectations). Herein, we present a new equation to estimate the selfing rate of individuals from a closed mating system:

$$\hat{s} = \frac{n_s}{N(1 - \beta_m)}, \quad (3)$$

where the associated variance is

$$\mathrm{Var}\,\hat{s} = \frac{\hat{s}[1 - \hat{s}(1 - \beta_m)]}{N(1 - \beta_m)}. \quad (4)$$

If there are x independent loci that show the same pattern, then the multilocus $\beta_m = .75^x$. As seen in Equation 4, the variance decreases as $1 - \beta_m$ approaches 1. However, more loci are needed to achieve a higher probability of discerning a selfed genotype in Method C compared to identifying a discernable outcrossed genotype in Method B (e.g., eight loci are needed to achieve a $1 - \beta_m = .90$). More details on some of the statistical properties and sample sizes needed to achieve precise estimates of $\hat{s}$ with Methods B and C are given in Fig. S1. For additional discussion on Cruzan et al. (1994) method of moments estimator, which our Methods B and C are related to, see Ritland (2002). We also note that Methods B

and C require loci to have Mendelian inheritance and in the case of multilocus estimates, to have independent assortment (i.e., recombination rate = 0.5). In most cases, we were able to directly verify Mendelian segregation and independent assortment using the methods described in Detwiler and Criscione (2011) (data not shown).

2.4.4 | Method D

In some situations, the $\hat{s}$ of a worm could be determined by combining Method A with Methods B or C. Worm A in Host 11 is such an example (Table S1). Here, locus di008 allowed unambiguous identification of outcrossed offspring of maternal Worm A with paternal Worm C, whereas two loci (di109 and di140) enabled Method B to estimate the outcrossing rate between maternal Worm A and paternal Worm B. The latter required accounting for genotypes at di109 or di140 that were created by an outcross between Worm A and C, but may have resembled an outcross between Worm A and B (see Table S1 for details). For Worm A, $\hat{s}$ was estimated as 1 minus the total outcrossing rate (i.e., the rate of C to A plus the rate of B to A). For Method D, offspring were resampled 10,000 times to obtain a bootstrap estimate of the variance.

2.5 | Significance tests of individual selfing rates

To determine whether individual tapeworm selfing rates differed from the expected value under random mating within a host, the proportion of simulated selfing rates ≤ selfing rate expected with random mating was calculated after 10,000 bootstraps over offspring. POPTOOLS v3.2.5 was used to perform the resampling (Hood, 2011). This proportion (or 1 minus the proportion if the proportion was >0.5) was multiplied by 2 to generate a two-tailed p-value. A $p < .05$ was considered significant. The expected selfing rate with random mating was based on the number of tapeworms within a host (i.e., $1/I_j$, where I_j is the infection intensity of host j). The bootstrap simulations were also used to calculate the variance and 95% confidence intervals of the individual estimates. Using exact binomial tests ($\alpha = .05$), we then asked whether more tapeworms had selfing rates that deviated (more or less) from random mating expectations than one expects by chance alone.

The above analyses treat each tapeworm as an independent test of the null hypothesis (i.e., the selfing rate within a host does not deviate from $1/I_j$). Recognizing that tapeworms within a host experience a common environment, we asked the same question at the level of host. Here, each tapeworm within a host is an independent replicate of the hypothesis in that host. To generate a p-value at the level of the host, we used the weighted Z-method to combine probabilities of individual tapeworms within a host (Whitlock, 2005). This test was run only on hosts where more than one tapeworm was tested. The inverse variance of the selfing-rate estimate of each tapeworm was used as the weight. As described above, binomial tests were conducted, but at the level of the host.

2.6 | Testing the relationship between selfing rate and intensity of infection

With random mating within hosts, the selfing rates of individual tapeworms are expected to be an inverse power function of the infection intensity experienced by those tapeworms. Thus, across tapeworms we expect the relationship $y = ax^b$, where y is the selfing rate and x is the intensity of infection. At an intensity of 1, the selfing rate is 100%; hence, we expect $a = 1$. The b parameter is predicted to be −1 with random mating within hosts; a value less negative would indicate more selfing than expected under random mating within hosts, whereas a value more negative would indicate less selfing. To test the above expectation, we fitted a linear model to the data ($\ln y = b^*\ln x + \ln a$) and estimated the parameters a and b.

As noted above, individual parasites within a host do not necessarily represent true replicates. Thus, to determine whether there was an inverse power relationship between infection intensity and selfing rates across all tapeworms, the data were modelled in two ways. (1) We averaged selfing rates within hosts (using the inverse of the variance of individual tapeworm selfing-rate estimates as a weight) and then fitted a general linear model (GLM) with the response (natural-log of the average selfing rate of tapeworms in a host) and explanatory variable (natural-log of the infection intensity). (2) We used a general linear mixed model (GLMM) that included the natural-log of the selfing rates for individual tapeworms as the response variable, natural-log of the infection intensity as the explanatory variable and Host ID as a random effect. Models were run using the STATS and LME4 (Bates, Machler, Bolker, & Walker, 2015a, 2015b) packages in R 3.2.2 (R Core Team, 2015), respectively.

The above analyses assume that the relationship between selfing rate and infection intensity is the same among the five sampled locations. Subpopulation could not be included as a factor in the analysis because there would not be sufficient power (due to too few samples per subpopulation across a range of intensities) to analyse the relationship between selfing rate and infection intensity. We note that both the gecko host and the tapeworm are genetically structured among the five locations in College Station (Detwiler & Criscione, 2014, 2017). However, if there were differences in the intensity to selfing-rate relationship among locations, we suspect it would be less likely that a relationship between intensity and selfing rate would be found because of excess noise in the data.

The data were also explored with nonlinear versions of these two models using untransformed data. Qualitatively, the data were robust as very similar best fit curves (and subsequent selfing-rate extrapolations, discussed below) were found with the nonlinear models. However, assumptions of either normality or homogeneity of variance were not met in the nonlinear models. Thus, all interpretations and extrapolations of selfing rates are restricted to the linear models using natural-log-transformed data.

2.7 | Estimating potential population selfing rates

Our goal here was to demonstrate how population-level selfing rates (s_p) based on the primary mating system (as opposed to an indirect means such as F_{IS}) could be generated from the results of the individual-based analyses. We refer to our estimates as "potential" population-level rates because density-dependent fecundity (a.k.a. the "crowding effect" in parasites; Read, 1951) could affect the selfing rates manifested at the population level (e.g., the realized proportion of metacestodes that are the product of self-mating in a natural population) independent of selection on inbred offspring themselves. There is abundant evidence that density-dependent growth and fecundity occur across many helminth taxa, especially tapeworms (Poulin 2007).

If there is no relationship between infection intensities (I_j) and individual selfing rates (s_{ij}, selfing rate of tapeworm i in host j), an estimate of s_p can be obtained by averaging the selfing rates of individuals ($s_p = \bar{s}_{ij}$) without a need to be concerned for density-dependent fecundity. When there is a relationship, assumptions need to be made about the variation in reproductive success among tapeworms. If there is random mating within hosts and assuming random reproductive success, s_p again equals $\bar{s}_{ij}$. However, an interesting relationship arises in recognizing that s_p can be calculated as a weighted average of the average selfing rates within hosts ($1/I_j$), where the weights are the proportion of worms in host j relative to the total number of tapeworms (I_j/N, where N is the total number of tapeworms). Hence,

$$s_p = \sum_{j=1}^{H} \left(\frac{1}{I_j}\right)\left(\frac{I_j}{N}\right), \quad (5)$$

where H is the total number of infected hosts. Equation 5 reduces to H/N, which is the inverse of the mean infection intensity. This simple result provides a baseline for understanding how the distribution of parasites can impact inbreeding. Assuming density-dependent fecundity, the weights can be altered. For example, high-intensity infections result in fewer offspring per parent tapeworm leading to large inequalities in reproductive success across all tapeworms from all hosts. Under such crowding effects, the effective population size of a parasite would be closer to the number of infected hosts rather than the number of parasites (Dobson, 1986). Following this logic, the weights would be $1/H$.

In our study, selfing rates were higher than within-host random mating expectations (i.e., $>1/I_j$), but there was still an inverse power relationship to infection intensities (see Results). Thus, we did the following to estimate potential population-level selfing rates from the five sampled populations for which we had intensity distribution data (Figure 2). First, because the a parameter was not significantly different than 1 in either model (see Results) and because biologically the selfing rate can only be 100% with an infection intensity of 1, we fixed $a = 1$ and re-estimated the b parameter in the two models (GLM and GLMM). We then used these predicted relationships (back-transformed to a power function) along with the distribution of tapeworms among hosts within each of the five locations to obtain two s_p estimates for each population. The first estimate assumed that there were equal chances of tapeworms contributing to the offspring pool (i.e., random variation in reproductive success); we refer to these as NC-based estimates for noncrowding conditions. Here, the proportion of tapeworms (from the total number of tapeworms sampled in a location) found in each intensity class (1 through the maximum intensity found in that subpopulation) was multiplied by the model-predicted selfing rates for that intensity and then summed across all intensity classes. The second estimate assumed density-dependent fecundity where the total number of parasite offspring originating from each host would be the same no matter the level of intensity; we refer to these as C-based estimates for crowding conditions. Here, the proportion of infected hosts at a given intensity class was multiplied by the model-predicted selfing rates for that intensity and then summed across all intensity classes. The above two assumptions of the variation in reproductive success likely represent the ends of a continuum.

To provide error ranges and to determine whether the NC- and C-based estimates led to statistically different signatures, we used Monte Carlo simulations. In each simulation round, the intensity to selfing-rate relationship $y = x^b$ was used with a bootstrapped distribution of infection intensities to calculate a selfing rate. For each round, a value of b was randomly picked from a normal distribution based on the mean and standard error in the GLM or GLMM estimations. Each round also consisted of a resampling with replacement of the infection intensities (i.e., the number of infected host remained constant) of the given population (infection intensity distributions are given in Figure 2). Thus, both model estimation error of individual tapeworm selfing rates and error in the distribution of infection intensities were incorporated into the overall error assessment of the NC- and C-based population-level selfing rates. We did 10,000 simulations and used percentile rankings of the simulated values to generate confidence intervals (CI). Because two population estimates with overlapping 95% CI may still be significantly different at $p = .05$ or $p = .01$ (Schenker & Gentleman, 2001), we used nonoverlapping 84% CI and 94% CI to assess whether the NC- and C-based estimates were significantly different at $p = .05$ and $p = .01$, respectively (MacGregor-Fors & Payton, 2013). The CI were constructed using the dPercentile function in POPTOOLS (Hood, 2011), which follows the algorithm recommend by the National Institute of Standards and Technology (e-Handbook of Statistical Methods, http://www.itl.nist.gov/div898/handbook, 2013).

3 | RESULTS

Parasite offspring at the metacestode stage were obtained from 52 adult tapeworms that originated from 22 hosts collected across five populations. A mean of 91.4 metacestodes (range 60–103) were genotyped per adult tapeworm (Tables 1–3). In total, 4,753 offspring were genotyped. Significance results for individual tapeworm selfing-rate estimates are given in Tables 1–3. We note that selfing rates

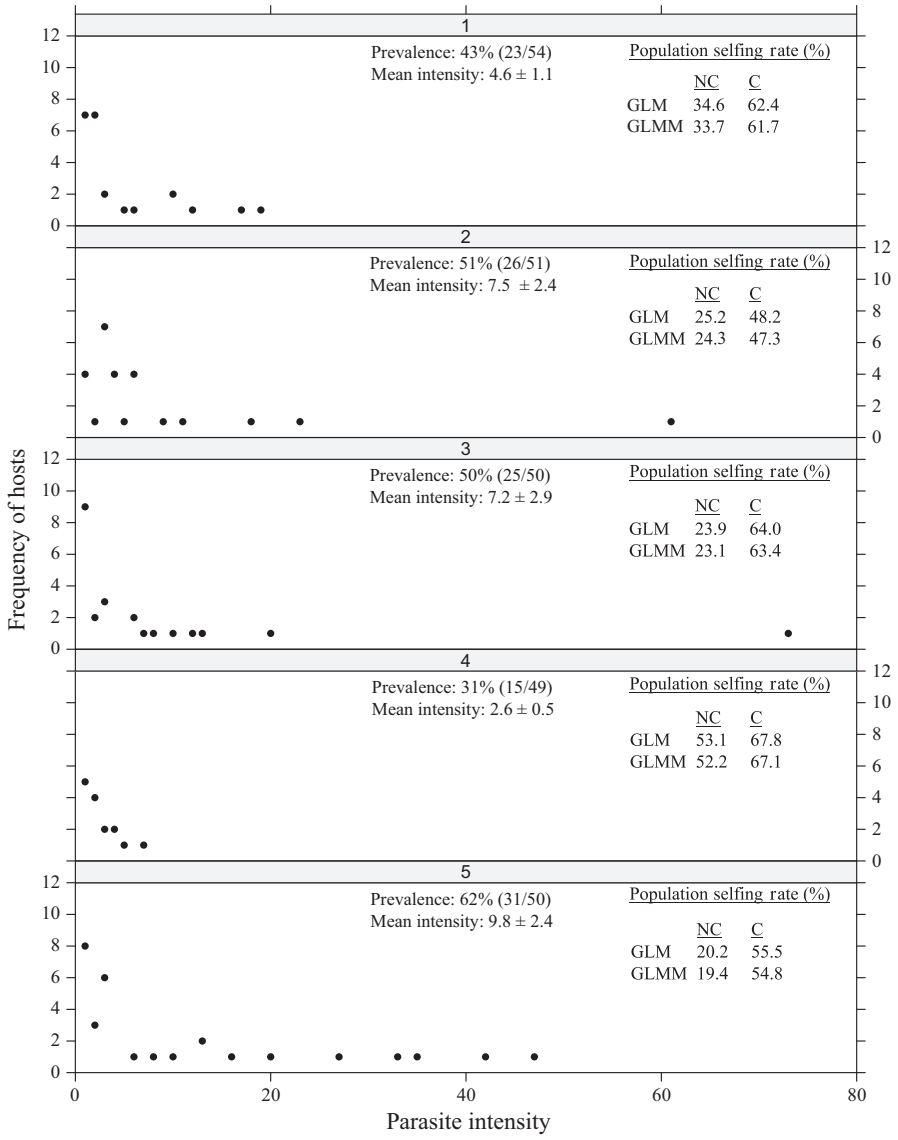

FIGURE 2 Distribution of infection intensities of *Oochoristica javaensis* among Mediterranean gecko hosts for each of the five populations. Prevalence of infection and mean intensity (±*SE*) is given for each location. Also included are the point estimates of the potential population-level selfing rates (given as percentages) estimated from the inverse power relationships of individual selfing rates to infection intensities (GLM and GLMM) combined with the distribution of parasites among hosts. NC-estimates (no crowding) were based on the assumption of random reproductive success of individual tapeworms, whereas C-estimates (crowding) were based on the assumption of density-dependent fecundity

could not be estimated for every tapeworm within a host because some tapeworms had identical genotypes, too few or no metacestodes were collected from the maternal tapeworm or the genotypes of the other adult tapeworms precluded a means to estimate the selfing rate. Table S1 gives the raw genotype data and the method of calculation for each tapeworm's selfing-rate estimate.

Overall, 69% (36/52) of the individual parasites significantly deviated from the selfing rate expected under random mating. There was more selfing than expected in 34 parasites and more outcrossing than expected in two parasites (Tables 1–3). Of the 16 parasites that did not have a selfing rate significantly different than that expected under random mating, 13 tended in the direction of more selfing. Binomial tests showed that more tapeworms had a significantly higher selfing rate than expected by chance alone ($p < .001$), whereas the number of tapeworms with higher outcrossing rates did not deviate from that expected by chance alone ($p = .74$). At the level of host, 15 of 17 hosts with more than one tapeworm had significantly more selfing and two hosts did not deviate from the expectation under random mating (Tables 1–3). A binomial test showed that more tapeworms at the level of hosts tested for a significantly higher selfing rate than expected by chance alone ($p < .001$).

There was a significant inverse power relationship between the average selfing rate within hosts and infection intensity in the GLM ($F_{1,\ 20} = 90.59$, $p < .0001$) where 82% (r^2) of the variance in selfing rate was explained by the model. The back-transformed equation, that is, the estimated inverse power function, was $y = 1.05\ x^{-0.65}$ (Figure 1). Confidence intervals (CI) for *a* included 1: 95% CI [0.87, 1.28], but for *b*, the values were >−1: 95% CI [−0.79, −0.51]. A similar significant relationship was also found with the GLMM (estimate of $b \pm SE = -0.60 \pm 0.10$, *t*-value = −5.97, $p < .0001$; Fig. S2). The variance explained was 42% and 44% for the fixed and fixed and random effect combined in the GLMM, respectively. The back-transformed equation was $y = 0.95\ x^{-0.60}$. Confidence intervals for *a* included 1: 95% CI [0.73, 1.24], but for *b*, the values were >−1: 95% CI [−0.79, −0.40].

Upon setting $a = 1$, the re-estimated inverse power functions were $y = x^{-0.613}$ (*b*, *SE* = 0.0237, 95% CI: −0.66, −0.56) and $y = x^{-0.632}$ (*b*, *SE* = 0.0333, 95% CI: −0.7, −0.57) for the GLM and

TABLE 1 Individual selfing rates of tapeworms found in hosts with an infection intensity of 2. The expected selfing rate under random mating within a host was 0.5 for each tapeworm

Host	Worm parent	N[a]	Selfing rate[b]	95% CI[c]	p-value[d]	Mating strategy[e]	p-value by host[f]
1	A	93	**.84** (.0017)	0.75–0.91	.0002	S	.0445
	B	93	**.24** (.0039)	0.13–0.37	.0004	O	
2	A	93	**.61** (.0026)	0.52–0.71	.0306	S	.1395
	B	95	.49 (.0026)	0.39–0.6	.9092	RM	
3	A	90	.67 (.0079)	0.50–0.85	.0638	RM	.1094
	B	93	.56 (.0034)	0.45–0.67	.3414	RM	
4	A	95	**.79** (.0018)	0.71–0.86	.0002	S	<.0001
	B	94	**.62** (.0025)	0.52–0.71	.0296	S	
5	A	93	**.63** (.0038)	0.51–0.74	.0498	S	.0190
	B	95	.65 (.0111)	0.46–0.87	.1308	RM	
6	A	90	**.72** (.0033)	0.60–0.82	.0002	S	.0012
	B	95	.43 (.0085)	0.26–0.63	.4968	RM	

[a]Number of genotyped offspring from the parent tapeworm.
[b]Bold indicates the individual tapeworm selfing rate is statistically different than expected under random mating. Variance of the estimate is given in parentheses.
[c]Confidence interval (CI) for selfing-rate estimate.
[d]*p*-values were calculated from the proportion of simulated selfing rates ≤ selfing rate with random mating after 10,000 bootstraps over offspring. The one-tailed probability was then converted to a two-tailed probability. The direction of significance is given in the Mating strategy column.
[e]The direction of significance based on the two-tailed test of the individual tapeworm mating system: S = significantly more selfing; O = significantly more outcrossing; and RM = random mating (i.e., not significantly different than the expectation based on the number of tapeworms within a host).
[f]The *p*-value by host was based on the weighted *Z*-method to combine probabilities of individual tapeworms within a host. If significant, it was always in the direction of significantly more selfing.

TABLE 2 Individual selfing rates of parasites found in hosts with an infection intensity of 3. The expected selfing rate under random mating within a host was 0.33 for each tapeworm

Host	Worm parent	N[a]	Selfing rate[b]	95% CI[c]	p-value[d]	Mating strategy[e]	p-value by host[f]
7	A	94	.41 (.0026)	0.32–0.51	.1072	RM	<.0001
	B	95	**.60** (.0025)	0.49–0.69	.0002	S	
	C	94	**.54** (.0026)	0.45–0.64	.0002	S	
8	A & B	*	*	*	*	*	N/A
	C	93	**.57** (.0055)	0.42–0.71	.0018	S	
9	A	94	**.78** (.0018)	0.69–0.86	.0002	S	<.0001
	B	94	**.97** (.0135)	0.75–1.19	.0002	S	
	C	89	**.77** (.0045)	0.64–0.89	.0002	S	
10	A	90	**.61** (.0027)	0.51–0.71	.0002	S	<.0001
	B	94	**.75** (.0020)	0.67–0.84	.0002	S	
	C	95	.39 (.0025)	0.29–0.48	.2464	RM	
11	A	93	**.18** (.0032)	0.07–0.29	.0060	O	.0018
	B	94	**.68** (.0023)	0.59–0.78	.0002	S	
	C	75	**.59** (.0032)	0.48–0.69	.0002	S	
12	A	82	**.51** (.0031)	0.40–0.62	.002	S	<.0001
	B	90	**.63** (.0026)	0.53–0.73	.0002	S	
	C	95	**.61** (.0025)	0.52–0.71	.0002	S	

Table contents (a–f) are as in Table 1. An asterisk indicates the selfing rate of these tapeworms could not be obtained for various reasons (see main text).

GLMM, respectively. Combining these functions with the distribution of parasites among hosts, we estimated population-level selfing rates assuming random reproductive success (NC-based estimates) or density-dependent fecundity (C-based estimates). The point estimates from the GLM and GLMM are shown in Figure 2. Figure 3 shows the GLM point estimates along with their 84% CI.

TABLE 3 Individual selfing rates of tapeworms found in hosts with infection intensities of 4 and above

Host	Worm parent	Intensity	N[a]	Selfing rate[b]	95% CI[c]	p-value[d]	Mating strategy[e]	p-value by host[f]
13	A	4	*	*	*	*	*	<.0001
	B		93	**.43** (.0060)	0.28–0.58	.0188	S	
	C		85	**.51** (.0029)	0.40–0.61	.0002	S	
	D		*	*	*	*	*	
14	A	4	94	.27 (.0020)	0.18–0.35	.7494	RM	.0264
	B		94	.34 (.0024)	0.24–0.44	.0542	RM	
	C		92	.23 (.0019)	0.14–0.32	.5164	RM	
	D		94	**.49** (.0027)	0.39–0.59	.0002	S	
15	A	4	93	**.59** (.0026)	0.49–0.69	.0002	S	<.0001
	B		95	.31 (.0022)	0.21–0.40	.2174	RM	
	C		93	**.35** (.0025)	0.26–0.45	.0360	S	
	D		*	*	*	*	*	
16	A	4	92	**.38** (.0026)	0.28–0.48	.0090	S	<.0001
	B		94	**.51** (.0026)	0.41–0.61	.0002	S	
	C		94	**.35** (.0027)	0.25–0.45	.0468	S	
	D		94	**.37** (.0029)	0.26–0.47	.0244	S	
17	A–D	6	*	*	*	*	*	N/A
	E		92	**.32** (.0049)	0.19–0.45	.0162	S	
	F		*	*	*	*	*	
18	A	6	60	.40 (.0236)	0.13–0.73	.1	RM	.0262
	B		92	.28 (.0044)	0.15–0.41	.0508	RM	
	C–F		*	*	*	*	*	
19	A–D	6	*	*	*	*	*	N/A
	E		92	.30 (.0125)	0.13–0.52	.15	RM	
	F		*	*	*	*	*	
20	A–C	7	*	*	*	*	*	N/A
	D		84	.30 (.0068)	0.13–0.46	.0738	RM	
	E–G		*	*	*	*	*	
21	A–E	8	*	*	*	*	*	N/A
	F		103	**.33** (.0029)	0.22–0.44	.0002	S	
	G–H		*	*	*	*	*	
22	A	9	95	**.34** (.0128)	0.13–0.59	.0204	S	<.0001
	B		84	.15 (.0015)	0.08–0.24	.2780	RM	
	C–D		*	*	*	*	*	
	E		85	**.32** (.0026)	0.22–0.42	.0002	S	
	F		87	**.22** (.0020)	0.14–0.31	.0064	S	
	G		92	**.24** (.0020)	0.15–0.33	.0014	S	
	H–I		*	*	*	*	*	

Expected selfing rates under random mating within a host were 0.25, 0.17, 0.14, 0.13 and 0.11 for tapeworms originating from infection intensities of 4, 6, 7, 8 and 9, respectively. Table contents (a–f) are as in Table 1. An asterisk indicates the selfing rate of these tapeworms could not be obtained for various reasons (see main text).

The GLM and GLMM produced nearly identical results where the GLM estimates (and their corresponding CI boundaries) were always higher by no more than 1 percentage point to the GLMM results (Figures 2 and 3). Estimates of population-level selfing rates among locations ranged from 19% to 53% when assuming equal contributions of offspring among tapeworms (Figures 2 and 3). The NC population-level selfing rates had a significant negative correlation to the mean infection intensities (Pearson correlation for both NC GLM and GLMM: $r = -.94$, $p = .017$). This correlation was expected given that the selfing rates were a direct function of the infection intensities. Assuming crowding effects, selfing rates among locations ranged from 47% to 68% (Figures 2 and 3). In contrast to

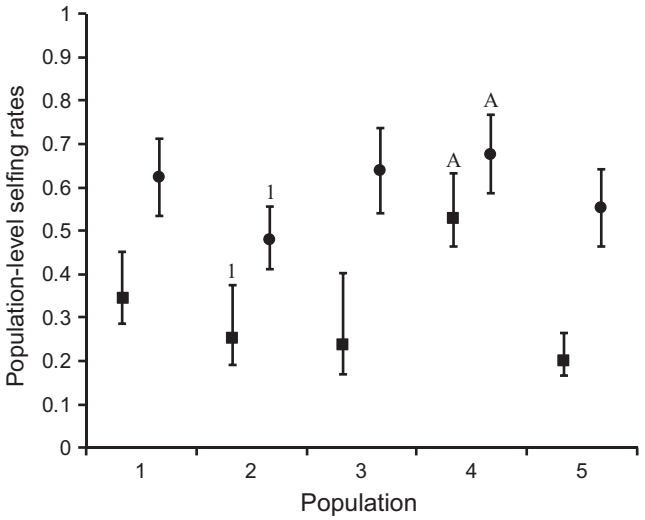

FIGURE 3 Potential population-level selfing-rate estimates and their 84% CI using the GLM results. Estimates using the GLMM results were nearly identical (all CI and point estimates shifted down by no more than 1.2 percentage points) and so are not shown. Squares are estimates based on the assumption of random reproductive success of individual tapeworms (NC), and circles are estimates based on the assumption of density-dependent fecundity (C). Letters above the bars denote that the 84% CI overlap (i.e., point estimates are not statistically different with a $p > .05$) between the NC- and C-based estimates. Numbers denote there is overlap at the 94% CI (i.e., point estimates are not statistically different with a $p > .01$)

the NC-based estimates, the correlation between selfing rates and mean infection intensities was no longer significant when assuming highly skewed reproductive success due to crowding effects (Pearson correlation for C GLM: $r = -.68$, $p = .20$, GLMM: $r = -.68$, $p = .21$). Within all populations, the point estimates of selfing were lower with the NC assumption relative to the C assumption (Figure 2). Judging significance at the .05 level, four of the five populations have estimates that would be statistically distinguishable between the NC- and C-based assumptions of reproductive success. Only in Population 4, where the fewest infected hosts were found and where infection intensities were no greater than seven tapeworms, was there overlap in the 84% CI. With a test value at .01, Populations 2 and 4 produced nonsignificant comparisons between the NC- and C-estimates (Figure 3).

4 | DISCUSSION

For the first time in nature, individual estimates of the primary mating system of a hermaphroditic flatworm parasite were generated. We found that individual tapeworms self-mated and outcrossed; hence, *O. javaensis* has a mixed-mating system. Based on the collective binomial test across individual tapeworm analyses (Tables 1–3) and that the *b* parameter of the inverse power function was >−1 (Figure 1), selfing rates were significantly greater than those expected from random mating within hosts. There are several evolutionary models that can explain the maintenance of a mixed-mating system (reviewed in Goodwillie et al., 2005) including some models that show stable equilibria with high selfing rates (e.g., Porcher & Lande, 2005). Our current study does not disentangle these various models, but biparental inbreeding, which this parasite exhibits (Detwiler & Criscione, 2017), could act to maintain a mixed-mating system (Ronfort & Couvet, 1995; Uyenoyama, 1986; but see Porcher & Lande, 2016). We also suggest a possible mechanical explanation that could lead to the elevated selfing rates above that expected from random mating. When dissecting the host, it was common to see tapeworms folded on themselves. Such folding may preclude some proglottids from outcrossing and result in "forced" self-mating among proglottids of a given tapeworm (analogous to geitonogamy in plants). With a certain proportion of proglottids prevented from outcrossing while the others have random mating, the selfing rate would be higher than that expected under complete random mating.

Regardless of the cause for the elevated selfing, the results of our study clearly demonstrated that the mixed-mating system of the gecko parasite *O. javaensis* is a significant function of the number of tapeworms in a host. Among-population studies in hermaphroditic plants have found negative correlations between population-level selfing rates and population densities (Eppley & Pannell, 2007 and references therein). Eppley and Pannell (2007) provided an explicit relationship to model individual selfing rates as a function of densities. Data from experimental plots provided support for their model showing a nonlinear decrease in selfing rates with increasing plant densities (the number of potential mates was held constant while interplant distances were decreased; Eppley & Pannell, 2007). Here, data from a natural population show a nonlinear decrease in individual selfing rates as a function of infection intensities, which largely reflect a change in density as the body sizes of collected hosts did not vary extensively. In particular, there was a significant fit to an inverse power function where a large proportion of the variance in individual selfing rates (82% and 44% in the GLM and GLMM, respectively) was explained by the intensity of infection (Figures 1 and S2). Importantly, because there was a significantly more selfing from that expected under random mating, the population-level selfing rate will no longer simply be the inverse of the mean infection intensity. Rather, the distribution of infection intensities among hosts will be needed to estimate population-level selfing rates. Thus, a major significance of our study's findings is that the distribution of hermaphroditic parasites among hosts can be a key driver in shaping a population's primary mating system, and hence the level of inbreeding in the parasite population.

Using the relationships between the selfing rates and infection intensities in conjunction with the observed intensities of parasites among hosts in each of the five locations (Figure 2), we were able to qualitatively evaluate how parasite distributions among hosts could impact the primary mating system at the population level. When assuming random reproductive success among individual parasites, our results show that infection intensities still have a direct impact on the average selfing rate. Even though the inverse of the mean

intensity is not the point estimate, low mean intensities lead to higher population-level selfing rates (NC-estimates in Figure 2). In contrast, with the assumption of density-dependent fecundity as a result of crowding, no significance was detected for a correlation between population selfing rates and mean intensities (C-estimates in Figure 2). For example, Population 3 had the same mean intensity of infection, yet it had a selfing rate over 15 percentage points higher than Population 2. Even populations with higher mean intensities could have relatively higher selfing rates (e.g., compare Population 5 to Population 2). The reason for the lack of a correlation is that crowding puts more weight (i.e., higher reproductive success) on tapeworms from lower infection intensities where they will experience higher selfing rates (Figure 1). For instance, in Population 3, 44% of the infected hosts had an intensity of 1, which accounts for 68% (0.44/0.64) of the selfing rate alone when assuming high density-dependent fecundity effects. These results indicate that crowding effects, a common ecological phenomenon among parasitic helminths (Poulin 2007), represent another means (via an indirect influence on reproductive success) by which infection intensities can influence population-level selfing rates. In effect, crowding can act to magnify the proportion of offspring that are the product of selfing and lead to statistically greater population-level selfing rates than when there is random reproductive success (Figure 3). However, our results do not necessarily indicate crowding effects will always result in a lack of correlation between mean intensities and population-level selfing rates as ultimately it depends on the underlying distribution of infection intensities. Based on the empirical distributions, our results provided a qualitative assessment of how infection intensities directly or indirectly influence population-level selfing rates. A future endeavour could focus on providing a formal analytical treatment of how parasite distributions influence flatworm mating systems when there are deviations from random mating within hosts.

In relation to the biology of our study system, density-dependent fecundity is conceivable. Crowding effects, where competition for limited resources within a host leads to reduced growth and reproduction, have been observed in tapeworms (Read, 1951), including morphological evidence of crowding in *Oochoristica* spp. (*O. bivitellobata*, Brooks & Mayes, 1976; *O. javaensis*, Criscione & Font, 2001a). Nevertheless, the NC- and C-based estimates of selfing represent potential rates that could be manifested at the population level and thus should be regarded as plausible hypotheses to be tested. In another study, pedigree reconstruction analysis (Wang, El-Kassaby, & Ritland, 2012) suggests that the selfing rates estimated with the assumption of density-dependent fecundity are more likely (Detwiler & Criscione, 2017).

How do our results compare to our general understanding of hermaphroditic parasite mating systems and can general conclusions be drawn about factors that influence the primary mating system? From 14 parasitic platyhelminth species included in Jarne and Auld (2006) the average selfing rate was 27% (SD = 0.29). Taken at face value, this level of selfing is comparable to the NC-based estimates, but is lower than the C-based estimates for *O. javaensis*. However, 12 of the studies included in Jarne and Auld (2006) estimated s indirectly via F_{IS}. We do not vet all these studies, but we draw attention to some caveats from two of the studies. In a study of the Asian tapeworm *Bothriocephalus acheilognathi*, eight microsatellite markers yielded an estimate of s = 0.418. But, all markers came from the internal transcribed spacer regions of the ribosomal DNA (Luo, Nie, Zhang, Yao, & Wang, 2003). Because these are not independent loci and because this is a multicopy gene that undergoes concerted evolution, it is not clear how to interpret the F_{IS} reported in this study. In the study on the marine trematode *Lecithochirium fusiforme*, Vilas, Paniagua, and Sanmartin (2003) found a high value of F_{IS}, and thus, a high estimate of s = 0.69 was obtained by Jarne and Auld (2006). Vilas et al. (2003) hypothesized that the high F_{IS} was due to a Wahlund effect. Indeed, a follow-up study found that cryptic genetic structure explained the high F_{IS} and that within each cryptic group there was random mating, that is, F_{IS} = 0 (Criscione, Vilas, Paniagua, & Blouin, 2011). Given these examples, we suggest that an overall reassessment of hermaphroditic parasite mating systems is in order although this is beyond the purview of our study.

At the time of Jarne and Auld (2006), the primary mating system of only two flatworm parasite species had been assessed via progeny-array methods (Lüscher & Milinski, 2003; Trouvé et al., 1996, 1999). Since then, two additional studies have been published (Rieger et al., 2013; Schelkle et al., 2012). All of these studies revealed that outcrossing and selfing is possible among the parasitic flatworms (cestodes, trematodes and monogenes) in an experimental laboratory setting. Nonetheless, the arduous task of conducting controlled experimental infections and/or the biology of the organism itself often presents difficulties in obtaining individual-based estimates of parasite selfing rates. For example, Lüscher and Milinski (2003) concluded that outcrossing was higher between tapeworms (*Schistocephalus solidus*) of similar body size. However, because offspring of paired tapeworms were collectively genotyped without regard to parent of origin, the reported average selfing rate could be misleading. An example illustrates this point. With parent genotypes of AA and BB and a pool of 80, 10 and 10 offspring of genotypes AA, AB and BB, respectively, the method of Lüscher and Milinski (2003) yields an "average" outcrossing estimate of 10%. Though, depending on the parent of origin of the AB offspring, the true average outcrossing rate could range from 5.6% to 25%. Thus, it is not clear how body size might influence the mating system. Though, a parental effects study on *S. solidus* (Benesh, 2013) found that size-matched individuals almost completely outcrossed.

The "Russian doll" mode of reproduction in monogenes of the genus *Gyrodactylus* also presents difficulties in obtaining individual estimates. The first two offspring born from a parent are the product of asexual reproduction with subsequent sexual reproduction (selfing or outcrossing) possible thereafter (Cable & Harris, 2002). Schelkle et al. (2012) report 3.7%–10.9% outcrossed genotypes after several generations of two experimental infections of *Gyrodactylus turnbulli*. Although it is clear that outcrossing occurs, it is not known what proportion of these outcrossed genotypes may have been asexually propagated.

Rieger et al. (2013) examined outcrossing rates among mixed clonal lines of the trematode *Diplostomum pseudospathaceum*.

Trematodes have obligate asexual reproduction in their first host prior to obligate sexual reproduction in their final host. They tested whether outcrossing exceeded that expected under random mating based on the assumption of equal proportions of mixed clonal line infections (e.g., 50% for two lines and 33% for three lines). The authors acknowledged infections may not take under equal proportions. Though, if this is the case, the null expectation is incorrect. Rather, the null expectation is dependent on the frequencies of each clone in a way analogous to calculating Hardy–Weinberg genotype proportions. For example, in fig. 4E of Rieger et al. (2013), the authors conclude the observed frequency of 57% of clone V genotypes in offspring indicates greater selfing of clone V (i.e., greater than the 25% expected under random mating given a 50% proportion in a dual-clone infection). However, this percentage of selfed genotypes can also be explained under random mating if the proportion of V clones in the host was actually about 75%.

Prior to our study, the work by Trouvé et al. (1996, 1999) on the trematode *Echinostoma caproni* provided the only individual-based selfing estimates of a flatworm parasite. Given the polymorphism limitations of allozyme markers, these studies were largely restricted to parasites from very distant locations (Mali, Egypt, Madagascar or Cameroon) to pair two individuals with distinct genotypes. Their work clearly shows that individual trematodes can outcross and self-mate, and in an interesting experiment where two trematodes from Mali were placed with a single fluke from Cameroon, the parasites from Mali preferentially outcrossed with one another (Trouvé et al., 1999). It will be interesting to test whether mate choice as verified with parentage data is a phenomenon that occurs within populations of a flatworm parasite.

The prior examples of progeny-array studies on flatworms all relied upon an experimental design where parents had a fixed genotype (Method A in our study). Method A can have limited utility for parasites collected from natural populations with low polymorphism, which will likely be the case in an inbred species such as *O. javaensis* (Detwiler & Criscione, 2017). While using parasites from divergent populations as in Trouvé et al. (1996, 1999) is a means to find fixed genotype parents, the results may not reflect the within-population dynamics, which is of primary evolutionary relevance. The Methods B and C we presented in this study to estimate individual selfing rates do not rely on fixed differences between parents. Thus, these methods could greatly facilitate experimental or field-based studies of various hermaphroditic animal species, especially species with low genetic diversity, and thereby increase the accessibility of different animal taxa (as molluscan systems currently predominate) for evolutionary studies dealing with hermaphroditic mating systems (e.g., sex role, Anthes, Putz, & Michiels, 2006; sex allocation, Scharer, 2009; inbreeding depression, Escobar et al., 2011).

In conclusion, we found a significant relationship between infection intensities and parasite selfing rates estimated from field-collected samples. Thus, this relationship is a direct reflection of the natural ecological dynamics experienced by *O. javaensis*. Given the extensive variation found among parasitic flatworm life histories, it is unlikely that this relationship will be universally applicable to all flatworm parasites. It will be interesting to determine whether particular parasite life histories relate to the occurrence or magnitude of an infection intensity to selfing-rate relationship.

Inbreeding is a powerful mechanism that can have wide-ranging evolutionary effects from genomes to populations (Charlesworth, 2003; Hartfield, 2016). There are progeny-array data for over 350 plant species and five decades of research on plant mating systems (Goodwillie et al., 2005). This foundational work is now allowing important questions to be addressed on how hermaphroditic mating systems impact genome evolution (Glemin & Galtier, 2012; Wright, Ness, Foxe, & Barrett, 2008). In contrast, there are progeny-array data for just five species of hermaphroditic flatworm parasites (including this study) (Criscione, 2016). Clearly, more work from diverse systems is needed to understand what ecological features of a parasitic lifestyle might influence hermaphroditic mating systems and hence inbreeding. Given the diversity of lifestyles and life cycles of parasitic flatworms, these systems are ripe for comparisons of how mating systems might influence genome evolution in parasites. The current study represents an effort to generate some of the foundational work needed among parasitic systems and provides methods that will facilitate field- or experimental-based studies related to hermaphroditic mating systems in parasites.

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DATA ACCESSIBILITY

All the data used in the analyses are included as Table S1 in Supporting Information.

AUTHOR CONTRIBUTIONS

J.T.D. and C.D.C. designed the research project. J.T.D. collected the hosts and parasites in the field and laboratory. J.T.D. and I.C.C. generated the genetic data. C.D.C. developed the methods for estimating the selfing rates. J.T.D., I.C.C. and C.D.C. performed analyses and wrote the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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