Received: 20 April 2017 | Revised: 2 June 2017 | Accepted: 2 June 2017

DOI: 10.1111/mec.14210

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Role of parasite transmission in promoting inbreeding: II. Pedigree reconstruction reveals sib-transmission and consequent kin-mating

Jillian T. Detwiler[1] | Charles D. Criscione[2]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

[2]Department of Biology, Texas A&M University, College Station, TX, USA

Correspondence
Charles D. Criscione, Department of Biology, Texas A&M University, College Station, TX, USA.
Email: ccriscione@bio.tamu.edu

Funding information
National Science Foundation, Grant/Award Number: DEB1145508

Abstract

Even though parasitic flatworms are one of the most species-rich groups of hermaphroditic organisms, we know virtually nothing of their mating systems (selfing or kin-mating rates) in nature. Hence, we lack an understanding of the role of inbreeding in parasite evolution. The natural mating systems of parasitic flatworms have remained elusive due to the inherent difficulty in generating progeny-array data in many parasite systems. New developments in pedigree reconstruction allow direct inference of realized selfing rates in nature by simply using a sample of genotyped individuals. We built upon this advancement by utilizing the closed mating systems, that is, individual hosts, of endoparasites. In particular, we created a novel means to use pedigree reconstruction data to estimate potential kin-mating rates. With data from natural populations of a tapeworm, we demonstrated how our newly developed methods can be used to test for cosibling transmission and inbreeding depression. We then showed how independent estimates of the two mating system components, selfing and kin-mating rates, account for the observed levels of inbreeding in the populations. Thus, our results suggest that these natural parasite populations are in inbreeding equilibrium. Pedigree reconstruction analyses along with the new companion methods we developed will be broadly applicable across a myriad of parasite species. As such, we foresee that a new frontier will emerge wherein the diverse life histories of flatworm parasites could be utilized in comparative evolutionary studies to broadly address ecological factors or life history traits that drive mating systems and hence inbreeding in natural populations.

KEYWORDS
biparental inbreeding, hermaphrodite, kin-mating, mating system, selfing rate, sib-transmission

1 | INTRODUCTION

To understand the role of inbreeding in genetically structuring natural populations (Charlesworth, 2003) and in the evolution of the mating systems themselves (Porcher & Lande, 2016; Ronfort & Couvet, 1995; Uyenoyama, 1986), it is necessary to have data on both the rates and ecological causes of self- and kin-mating in nature. Among free-living hermaphroditic species, kin-mating in addition to self-mating can be a significant contributor to population levels of inbreeding (Griffin & Eckert, 2003; Herlihy & Eckert, 2004). In particular, in species with limited dispersal (e.g., plant species where seeds drop next to their natal parent; Vekemans & Hardy, 2004), related individuals may be predisposed to mating with one another. In an analogous way to plant seed dispersal mechanisms, the life cycle patterns of some animal parasites may lead to the cotransmission of sibling parasites and subsequently predispose them to kin-mating (Anderson, Romero-Abal, & Jaenike, 1995; Nadler, 1995). Yet, empirical mating system studies on hermaphroditic flatworm parasites are scarce and

largely limited to indirect inference using the inbreeding equilibrium (i.e., the level of inbreeding reached under a constant system of mating across generations; Hedrick & Cockerham, 1986) relationship of F_{IS} (estimated from deviations of Hardy–Weinberg genotype frequencies) to the selfing rate (s), that is, $F_{IS} = s/(2\text{-}s)$ (reviewed in Jarne & Auld, 2006; Detwiler, Caballero, & Criscione, 2017). Critically, there are no estimates of kin-mating rates, and thus, the contribution of biparental inbreeding to F_{IS} is unknown in flatworm parasites.

For many metazoan parasites of animals, especially endoparasites, adult breeders exist in a closed mating environment bounded by an individual definitive host, that is, a parasite infrapopulation wherein parasite sexual reproduction occurs (Bush, Lafferty, Lotz, & Shostak, 1997). Parasite offspring pass into the external environment where progeny from different parental infrapopulations may or may not mix prior to infection of definitive hosts, and then once again, adult breeders will end up separated among hosts. The degree of offspring mixing could be a function of various ecological, life history or physiological traits of the parasite and its host. This transient separation of breeders into infrapopulations is repeated for each generation (Criscione & Blouin, 2005). Simulation modelling has shown that inbreeding measured across all parasites within a host population, that is, a parasite component population (Bush et al., 1997), increases with clumped sibling transmission (Cornell, Isham, Smith, & Grenfell, 2003; Dharmarajan, 2015).

Inference of sibling parasite transmission is commonly assessed by testing whether genotype frequencies are nonrandomly distributed among individual hosts, for example, estimating parasite F_{ST} among hosts or average within-host–parasite relatedness (reviewed in Criscione, Poulin, & Blouin, 2005; Gorton, Kasl, Detwiler, & Criscione, 2012). While *F*-statistics and relatedness/kinship estimators provide useful tools for determining whether there is nonrandom parasite transmission among hosts within a component population sample (Gorton et al., 2012; de Meeûs et al., 2007), there are limitations in quantifying cotransmission and kin-mating rates. For example, Wang (2014) discusses how the magnitude of kinship estimators is more or less arbitrary when reference allele frequencies are calculated from the current sample. Thus, average within-host kinship values may not be comparable among different component population samples or species because a kinship value may not equate to relationship status, for example, a value of 0.25 may not indicate full-sibs. A similar problem potentially exists in comparisons of the magnitude of among-host F_{ST} across different populations or species if marker loci are affected substantially by mutations (Wang, 2015).

As an alternative, pedigree reconstruction methods have been advocated as a means to directly assess the degree to which sibling parasites are cotransmitted (Criscione et al., 2005). In this study, we propose that pedigree reconstruction data can also provide empirical estimates of potential kin-mating rates. Given that many metazoan parasites of animals exist in closed mating systems, the percentage of highly related dyads (half- and full-sibs) within hosts can be used to estimate potential kin-mating rates. With the recent advent of new pedigree reconstruction methods, especially for hermaphroditic species that can self-mate (Wang, El-Kassaby, & Ritland, 2012; Wang & Santure, 2009), it is now possible to use this more direct approach to provide comparable estimates of sibling cotransmission and potential kin-mating rates. Moreover, pedigree reconstruction data can be used to estimate the proportion of individuals that are the product of self-mating, and hence provide an estimate of a realized selfing rate (i.e., an estimate based on the selfed individuals that survived to the sampled stage) that directly contributes to F_{IS} (Wang et al., 2012).

In our study, we analysed levels of inbreeding within five component populations of the hermaphroditic tapeworm *Oochoristica javaensis*. This parasite has a two-host terrestrial life cycle that involves an arthropod intermediate host and a lizard definitive host, the Mediterranean gecko *Hemidactylus turcicus* (Criscione & Font, 2001a,b,c). Both the host and parasite are considered introduced species and occur commonly on human structures throughout the southern U.S.A. (Criscione & Font, 2001a,b,c; Detwiler & Criscione, 2011). Given the life history traits of both the gecko host and *O. javaensis*, we hypothesized there would be a strong propensity for cosibling transmission and thus a major role for kin-mating in contributing to overall levels of inbreeding. For example, adult Mediterranean geckos are territorial and mark–recapture studies have shown that adult geckos have ranges within a few metres (Selcer, 1986). Thus, faecal deposits are likely concentrated. Moreover, the tapeworm gravid proglottids, each of which can contain well over 100 parasite larvae (CDC, unpublished), are released in the faeces intact. Thus, an arthropod intermediate host will likely ingest many sibling parasites. Subsequently, parasite offspring will be transmitted as a clump when an infected intermediate host is ingested by a gecko definitive host.

In Detwiler et al. (2017), we found that transmission of *O. javaensis* in terms of infrapopulation infection intensities had an inverse power relationship to the selfing rates of individuals. Using this relationship to estimate potential population-level selfing rates required assumptions about the variance in reproductive success among parasites. Thus, our first goal of the current study was to test whether the realized selfing rates estimated from pedigree reconstruction analyses were better explained by the potential population selfing rates that assumed random reproductive success or that assumed density-dependent fecundity (i.e., crowding effects). Our second goal was to examine how another aspect of transmission, that is, whom you are transmitted with, could contribute to inbreeding across the component population. In particular, we asked whether sibling parasites co-occurred within infrapopulations more often than expected given the percentage of related dyads across the component population. We also showed how the percentage of related dyads within hosts can be used to estimate potential kin-mating rates, and in doing so, provided the first kin-mating rate estimates for a hermaphroditic parasite. Subsequently, we found that the combined but independently estimated realized selfing rates and potential kin-mating rates explained the observed component population F_{IS} values. 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 flatworm parasites have been hampered by the inherent difficulties in generating progeny-array data from field samples. Hence, we also discussed how pedigree reconstruction data and the methods we employed could be used for hermaphroditic parasites, or more generally any species with a closed mating system, to assess mating systems and the factors that drive inbreeding in natural populations.

2 | MATERIALS AND METHODS

2.1 | Field collections

Details on host and parasite sampling including descriptions of collection locations are given in Detwiler and Criscione (2014) and Detwiler et al. (2017). In short, we collected from five locations in College Station, Texas, USA Each of these locations represented random-mating gecko populations, but with significant genetic differentiation between all pairs of gecko populations and an overall F_{ST} = 0.14 (see Detwiler & Criscione, 2014). We will report on host–parasite costructure analyses elsewhere, but here we simply note that there was also significant differentiation for the parasite between all pairs of locations with an overall F_{ST} = 0.15 (unpublished data). These patterns indicated that each of the five locations were largely independent from one another and thus, we treated them as such (i.e., separate component populations) for the remainder of the study.

2.2 | Genetic markers

DNA extraction and microsatellite genotyping followed that of Detwiler and Criscione (2011). The scolex and neck region of individual tapeworms were used for DNA extractions. We used 12 microsatellite markers (di008, di019-1, di030, di033, di035, di068, di097, di109, di131-2, di140-1, tri001 and tet012-1) that were described in Detwiler and Criscione (2011). Approximately 5% of individuals were genotyped twice, and no discrepancies in allele calls were found. Our previous work on this system (Detwiler & Criscione, 2011; Detwiler et al., 2017) revealed that all the above-mentioned 12 markers showed Mendelian inheritance and that, where testable, there was no evidence for physical linkage among loci, although this was only testable for about half the pairwise combinations of loci (see Detwiler & Criscione, 2011 for methods on tests of Mendelian inheritance and independent assortment).

2.3 | Characterization of overall genetic diversity and inbreeding

Within each of the five component populations, the number of alleles, observed heterozygosity (H_o) and gene diversity (H_s) were calculated for each locus with FSTAT v2.9.3 (Goudet, 1995). Significance for single locus and multilocus F_{IS} estimates (Weir & Cockerham, 1984) was assessed with a two-tailed test based on 10,000 randomizations of alleles among individuals using SPAGEDI v1.5 (Hardy & Vekemans, 2002). To obtain confidence intervals (CI) for the multilocus estimates of F_{IS}, we used GENETIX v4.05 (Belkhir, Borsa, Chikhi, Rausfast, & Bonhomme, 2004) to generate 10,000 bootstrap (over individuals) values. Confidence intervals of varying widths (discussed below) were generated for the multilocus estimates of F_{IS} and for additional statistics described below. To be consistent in CI construction, we used 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). INSTRUCT (Gao, Williamson, & Bustamante, 2007) was used to generate Bayesian estimates of component population inbreeding coefficients (Table S1), but because the point estimates were nearly identical to the Weir and Cockerham (1984) multilocus estimates (Table 1), we focused on the latter for the remainder of the study.

Linkage disequilibrium (LD) was assessed using the genotypic disequilibrium tests implemented in GENEPOP (Rousset, 2008). Tests were conducted between pairs of loci within sampling areas with 5,000 dememorizations, 5,000 batches and 5,000 iterations. We tested for an overall pattern of LD within each component population using an exact binomial test (α = 0.05) to determine whether the observed number of significant pairwise tests was greater than expected (Waples, 2015). For each component population, we did these tests across all loci pairs and then split the data into two groups according to the loci we knew independently assorted to those for which we had no information on independent assortment. We then used a Fisher's exact test to determine whether there was a difference in the number of significant LD pairs between these two groups.

2.4 | Pedigree reconstruction methods

We used the full-likelihood method of sibship reconstruction, which also estimates the proportion of individuals that are the product of selfing events, implemented in the software COLONY v2.0.6.2 (Jones & Wang, 2010). Details of the method are given in Wang and Santure (2009) and Wang et al. (2012). For each component population data set, the following settings were specified in COLONY: female and male polygamy with inbreeding for a monoecious species, length of run was very long under the full-likelihood method with very high precision, three runs, allele frequencies estimated from the data set and were updated as the analysis was run, and sibship scaling was set to yes. No sibship prior was used as individual tapeworms can have offspring numbers in the low thousands (CDC, personal observations). Allelic dropout rate was set to 0.001 and mutation/error rate was set to 0.001 as genotype scoring was unambiguous, and our results suggested a very low influence of technical errors in general (see Results). These sibship reconstruction methods assume that the sample does not contain individuals from different generations (e.g., parents and offspring). We believe discrete generations are a reasonable assumption in this system given the parasite's life cycle pattern (Criscione & Font, 2001a), relatively short host lifespan (about 3 years, Selcer, 1986) and near identical body sizes of worms within hosts

TABLE 1 Sample sizes (*N*) and multilocus estimates of F_{IS} (Weir & Cockerham, 1984) with bootstrap 95% confidence intervals (CI) for each of the five component populations of *Oochoristica javaensis*

Population	Host *N*	Infected hosts *N*	Collected parasites *N*	Genotyped parasites *N*	F_{IS}	95% CI
1	54	23	106	104	0.615	0.540–0.681
2	51	26	194	190	0.572	0.502–0.634
3	50	25	179	177	0.511	0.433–0.583
4	49	15	39	39	0.718	0.578–0.830
5	50	31	304	298	0.489	0.428–0.547

(unpublished data), which suggest concurrent infections. The percentages of related dyads (full- and half-sibs; collectively referred to as kin dyads) were of primary interest in our study. A series of simulations that were conducted in COLONY (Wang, 2013) showed there was little to no bias in the estimation of the overall percentage of kin dyads when using parameters reflective of our data sets (Table S2).

2.5 | Evaluating pedigree reconstruction selfing rates

Using the best sibship configuration from the full-likelihood method, an individual was classified as the product of a selfing event if both its parents had the same identifier. The COLONY -derived point estimate of the selfing rate is the proportion of the sampled individuals that are classified as the product of a selfing event in the best configuration. COLONY also provides 95% CI for the selfing rate based on a sum of the variance due to sampling error (binomial variance based on the total number of sampled individuals) and variance due to estimation error (Wang et al., 2012). The square root of this combined variance (i.e., the standard deviation) is multiplied by 2 to generate a normally approximated CI (J. Wang, personal communication). The estimation error is generated from other plausible configurations with relatively high likelihood values. These plausible configurations, which are stored in the "archive" file in COLONY, may have the same or different selfing-rate estimates (or other estimated parameters such as the numbers of full and half-sibs). The COLONY manual describes how to calculate the probabilities of these other configurations.

To be consistent in CI comparisons for statistics of interest in our study, we used Monte Carlo simulations to simultaneously generate CI. In this section, we describe our method to get the selfing-rate CI and in sections below, we describe the means to obtain the CI of other statistics such as the percentages of related dyads. Our simulations also included estimation and sampling error. First, the probability of each individual configuration in the archive file was calculated. In each simulation round, a configuration, and hence its selfing-rate estimate, was randomly selected based on its probability using the DiscreteDev function in POPTOOLS. This first step accounts for the estimation error. Next, this selected selfing rate was used as the probability to randomly select a number of selfed individuals based on the binomial sampling error (given the sample size of that population). This step, which incorporates the sampling error, was performed with the dBinomialDev function in POPTOOLS. After 10,000 simulations of the selfing rate, CI were generated with the dPercentile function in POPTOOLS. We emphasize these simulations accurately estimated the total variance as discussed above; however, our simulated selfing-rate CI may differ slightly (typically less than one percentage point at either boundary) from the normally approximated COLONY CI (data not shown).

In our prior study, we used an inverse power relationship between infection intensities and individual tapeworm selfing rates along with the distributions of parasites among hosts to provide hypotheses of potential population-level selfing rates (Detwiler et al., 2017). These estimates were generated assuming either random reproductive success among parasites (NC-estimates for noncrowding) or density-dependent fecundity (C-estimates for crowding). To determine whether the realized selfing rates from the pedigree reconstruction analyses (hereafter referred to as COLONY-selfing rates) reflected either of these extrapolated estimates, we compared CI overlap as a means to test whether the COLONY-selfing rates were statistically different from the NC- or C-based estimates. For these comparisons, we used the NC- and C-based estimates and their CI generated under the general linear mixed model (GLMM) in Detwiler et al. (2017). Nonoverlapping 84% CI and 94% CI were used to assess significance at $p = .05$ and $p = .01$, respectively (MacGregor-Fors & Payton, 2013). We used an overall approach to determine whether the COLONY-selfing rates could be explained by either the NC- or C-based estimates. For example, under the null hypothesis that the COLONY-selfing rates were no different than the NC-based estimates across the five component populations, then no more than one comparison is expected to show nonoverlapping 84% CI. That is, with five tests and $\alpha = 0.05$, the exact binomial probability of two or more significant tests is 0.023.

2.6 | Assessing cosibling transmission and subsequent potential for kin-mating

For kin-mating to cause an increase in F_{IS}, outcrossing between related individuals needs to occur at a frequency greater than expected by chance alone. As metazoan endoparasites exist in closed mating systems, the potential for biparental inbreeding can be directly assessed by testing for a higher percentage of kin dyads within hosts compared to the percentage of kin dyads across the entire component population (i.e., the expectation based on random chance). This comparison is in itself a direct test of cosibling parasite transmission.

For each component population, dyads across an entire component population were counted as full-sibs, half-sibs or unrelated based on the parent identifications (ID) given in the best configuration. For example, if two offspring had the single parent ID #1 (i.e., full-sibs from a selfing event) or both had the parents #1 and #2, they were classified as full-sibs. If one offspring had parent #1 and the other offspring had parents #1 and #2 (i.e., selfed-outcrossed sibs), or if one offspring had parents #1 and #2 and the other offspring had parents #1 and #3, they were classified as half-sibs. The number of kin dyads over the number of possible dyads across the entire sample is the frequency point estimate for the random chance expectation of outcrosses between kin dyads (P_E). Similar counts of full-sibs, half-sibs and unrelated were made for dyads that co-occurred within the same hosts. Because outcrossings are confined to discrete mating groups (i.e., hosts), the percentage kin dyads within hosts (P_K) is calculated as a weighted average over hosts with intensities >1 as infections with single tapeworms do not contribute to outcrossing. The weights are the proportion of tapeworms within a given host relative to the total number of tapeworms that occur in intensities >1. Calculations are made separately for full- (P_F) and half-sibs (P_H) within hosts, but for simplicity, we continue to refer to them collectively as the percentage of kin dyads within hosts ($P_K = P_F + P_H$).

To determine whether P_K was greater than P_E, we used the simulation protocol described above to provide CI for both metrics. In each simulation round, an archived configuration was randomly selected based on its probability to account for pedigree estimation error. Using the selected pedigree configuration, full-sib, half-sib and unrelated parasite dyad counts were made within and between hosts. Because P_K is shaped by the discrete mating boundaries, host individuals were considered the sampling unit. Thus, the next step was to resample with replacement the individual hosts (along with their within- and between-host–parasite dyad counts and infection intensities) to account for sampling error. Using this bootstrapped sample, P_K and P_E were calculated. We note to recover the correct value of P_E, the between-host dyad counts are halved to account for reciprocal parasite pairs between any two hosts and bootstraps are conducted over all infected hosts. Using 10,000 simulated values of both P_K and P_E, CI were generated to determine whether P_K was greater than P_E.

We also used a more traditional analysis of assessing nonrandom transmission among hosts to provide a basis of comparison between the different approaches and past studies. In particular, average within-host Ritland and Loiselle pairwise kinship coefficients were computed in SPAGEDI v1.5 (Hardy & Vekemans, 2002; Vekemans & Hardy, 2004) where 10,000 randomizations of parasites among hosts were conducted to assess significance. These estimators were calculated and tested separately for each component population using the respective allele frequencies of the given component population as the reference.

In Detwiler et al. (2017), we found that selfing rates were associated with infection intensities. Similarly, we wanted to determine whether cotransmission of related parasites, and hence the opportunity for biparental inbreeding, was associated with intensities of infection. Therefore, we tested for a relationship between individual host infection intensities and percentage of kin dyads within the hosts (i.e., P_K per host). P_K per host was the response variable. Host infection intensities, component population and the interaction between infection intensity and component population were explanatory variables. Values of P_K per host came from the results of the best configuration. To meet the assumption of homogeneity of variance, P_K was ln(x+1)-transformed and intensity ln-transformed. Normality could not be met, so we used permutation-based ANCOVA as implemented in the *lmPerm* (Wheeler & Torchiano, 2016) package in R 3.3.3 (R Core team, 2017). Note, we report traditional *F*-values, but use permutation *p*-values to assess significance.

Similar tests were conducted with the Ritland and Loiselle kinship coefficients where the average pairwise kinship coefficients for parasites within each individual host were calculated. These values from each infrapopulation were then regressed on each host's respective infection intensity. Analyses were conducted separately for each component population using *lmPerm*. A combined analysis with population as a factor could not be used to evaluate the above because the kinship values are not comparable among the populations due to different allele frequency references (Wang, 2014).

2.7 | Estimating potential kin-mating rates and accounting for levels of inbreeding

We found strong support for the cotransmission of related parasites at a higher frequency than expected by chance within each component population (see Results). Hence, in addition to the self-mating, there is the potential for kin-mating to contribute to the levels of inbreeding in each component population. To determine how much selfing alone and selfing combined with potential kin-mating could contribute to inbreeding, we used the general inbreeding equilibrium formula that accounts for mixed percentages of self-mating, full-sib mating and half-sib mating (Hedrick & Cockerham, 1986):

$$F_e = \frac{\sum_j \frac{S_j}{2^j}}{1 - \sum_j S_j \left[1 - \left(\frac{1}{2}\right)^j\right]}$$

S_j represents the percentage of matings for a particular relationship category: self-mating is classified as S_1, full-sibs are S_2 and half-sibs are S_3 (eq. 15a in Hedrick & Cockerham, 1986). This equation reduces to $F_e = s/(2 - s)$ when there is only self-mating ($s = S_1$).

A major advantage of closed mating systems is that possible outcrosses can be quantified. In particular, P_F and P_H provide estimates of the percentages of potential outcrosses that are between full- and half-sibs, respectively. Subsequently, P_F and P_H can be used to estimate the potential kin-mating rates S_2 and S_3, respectively. For simplicity, we refer to an overall kin-mating rate, $t_K = S_2 + S_3$, but note that in calculating F_e, we use estimates for S_2 and S_3.

The total outcrossing rate ($t_T = 1 - s$) can be broken down into $t_T = t_K + t_U$, where t_U is the unrelated mating rate. The proportion of

outcrosses that are due to kin-mating, $t_K/(t_K + t_U)$, can be estimated with P_K assuming that actual outcrosses occur randomly within infrapopulations. We return to this assumption in the Discussion. Hence, $(1 - s) * P_K = t_K$ was separated into components, $(1 - s) * P_F = S_2$ and $(1 - s) * P_H = S_3$. As an estimate of s, we used the COLONY-selfing rate.

In the prior section, we estimated P_K as a weighted average based on the infection intensities. This is the appropriate weight if one simply wishes to know whether kin dyads co-occur, and thus possibly mate more frequently than expected by chance. The above calculations for kin-mating rates, however, represent "potential" kin-mating rates because we are using the frequencies of possible matings as a means to infer the proportion of offspring resulting from kin-mating. Hence, weighting by infection intensities carries the assumption of random reproductive success. Because P_K per host was not related to infection intensities (see Results), then density-dependent fecundity is not of concern in our system and the current weighting remains appropriate (see also Detwiler et al., 2017, where this issue is discussed in relation to estimating potential population-level selfing rates). We return to this subject in the Discussion.

To obtain CI around F_e based on COLONY-selfing rates alone, F_e based on COLONY-selfing rates and potential kin-mating rates (S_2 and S_3 accounted for), and overall potential kin-mating rates themselves (t_K), calculations were carried out in each round of the simulations described previously. Point estimates were based on the best sibship configuration. Subsequently, we compared 84% and 94% CI to ask whether F_e based on COLONY-selfing rates alone or if the F_e based on COLONY-selfing rates and potential kin-mating rates could explain the observed F_{IS} values in each component population.

3 | RESULTS

3.1 | Field collections

From 254 geckos collected across the five locations, a total of 822 tapeworms were found. The vast majority of tapeworms (n = 808; 98.3%) were successfully genotyped across all loci (Table 1). Fourteen individuals were excluded because either the scolex was lost in the processing or because of poor template quality. Figure 2 in Detwiler et al. (2017) shows the parasite intensity distributions for each of the five component populations.

3.2 | Characterization of overall genetic diversity and inbreeding

Overall, there was low genetic diversity for each locus and locus tri001 was polymorphic only within Population 1 (Table S3). Average number of alleles per polymorphic locus ranged from 2.2 to 2.5, and average H_s ranged from 0.29 to 0.51 per polymorphic locus across the component populations.

Multilocus F_{IS} estimates were high and significant in each of the five component populations thus indicating substantial inbreeding within each of the parasite component populations (range: 0.49–0.72, Table 1). Within each component population, there was consistency across loci in showing high and significant F_{IS} values (Table S3). Only in Population 4, which had the smallest sample size (n = 39), did two loci test nonsignificant. However, the F_{IS} values of these two loci were still 0.29. The high consistency among loci coupled with Bayesian estimates of F (Table S1) that were nearly identical to the Weir and Cockerham (1984) multilocus estimates (Table 1) suggests technical artefacts (e.g., null or false alleles) had little influence on the measures of inbreeding in the data sets. Genotype data are provided in Table S4.

Results of LD analyses are given in Table S5. Overall, there was evidence of LD. The percentage of pairwise comparisons that were significant within each location ranged from 45.5 to 100% (an exact binomial test was highly significant in each component population, all p-values <.0001, Table S5a). Splitting the data between loci we knew were physically independent to those for which we had no information on independent assortment (Table S5b), which showed both sets of data were no different in terms of the proportion of loci pairs that tested significant (all Fisher's exact p-values >.25, Table S5c). Given that the pairs of loci for which we do not have assortment data show no more of a tendency to have LD than the pairs of loci for which we know are physically independent (i.e., not linked), we assumed that the overall LD patterns were a reflection of population history (e.g., founder events coupled with nonrandom mating as discussed below) rather than physical proximity of loci or selective influences.

3.3 | Evaluating pedigree reconstruction selfing rates

Point estimates of the COLONY-selfing rates are given in Table 2. Comparisons of the realized component population-level selfing rates from the pedigree reconstruction analyses to the potential NC- or C-selfing-rate estimates are shown in Figure 1. All NC-estimates were lower than the COLONY-selfing rates. Judging significance at $p < .05$ and $p < .01$, there are four and two rejections of five tests, respectively, when comparing the COLONY-selfing rates to the estimates based on the assumption of random reproductive success (NC). Under a null hypothesis of no difference and $\alpha = 0.05$ or $\alpha = 0.01$, the exact binomial probabilities of observing these number of rejections are $p = .00003$ and $p = .00098$, respectively. In stark contrast, when comparing the COLONY-selfing rates to the C-estimates (assumes density-dependent fecundity), there is only one rejection of five tests at $p < .05$ and no rejections at $p < .01$. Under a null hypothesis of no difference and $\alpha = 0.05$ or $\alpha = 0.01$, the exact binomial probabilities of observing these number of rejections are $p = .23$ and $p = 1$, respectively.

3.4 | Assessing cosibling transmission and subsequent potential for kin-mating

Table 2 provides the best configuration point estimates for the numbers of full-sib, half-sib and unrelated dyads in each component

TABLE 2 Sibship reconstruction results based on the best configuration and subsequent point estimates of the percentages of kin dyads within hosts and overall, realized selfing rates, potential kin-mating rates and F_e

	Population 1		Population 2		Population 3		Population 4		Population 5	
	In host	Total	In host	Total	In host	Total	In host	Total	In host	Total
Full-sibs[a]	76	124	425	520	221	354	37	65	1089	3324
Half-sibs[a]	156	379	349	732	1134	1572	5	34	806	4316
Unrelated[a]	238	4853	1593	16703	1714	13650	11	642	1958	36613
P_F[b]	25.75		17.99		10.97		51.96		25.26	
P_H[b]	27.49		18.97		32.38		15.69		20.93	
P_K and P_E[b]	53.24	9.39	36.97	6.97	43.36	12.37	67.65	13.36	46.19	17.26
S_1[c]	50.96		53.16		45.20		74.36		50.34	
S_2[d]	12.63		8.43		6.01		13.32		12.55	
S_3[d]	13.48		8.89		17.75		4.02		10.39	
t_K[d]	26.11		17.32		23.76		17.35		22.94	
F_e[e]	0.569		0.502		0.459		0.832		0.526	

[a]Relationship categories based on the best configuration output of COLONY. Given for each component population are the numbers of kin or unrelated dyads that occurred within hosts (In host) and over the entire component population (Total).
[b]P_F and P_H are calculated as weighted averages across hosts (see main text) and are the proportions of full- and half-sib dyads within hosts, respectively. $P_K = P_F + P_H$. P_E is the sum of total full- and half-sib dyads divided by the total number of possible dyads overall. Values are expressed as percentages.
[c]S_1 is the estimate of the realized selfing rate (s) based on the pedigree reconstruction analysis in COLONY. Values are expressed as percentages.
[d]S_2 and S_3 are the full- and half-sib potential kin-mating rates calculated as $(1 - s) * P_F$ and $(1 - s) * P_H$, respectively. t_K is the overall potential kin-mating rate ($S_2 + S_3$). Values are expressed as percentages.
[e]F_e was calculated using eq. 15a in Hedrick and Cockerham (1986) using the realized selfing rates (S_1) and potential kin-mating rates (S_2 and S_3).

population as a whole and within hosts. P_E ranged from 7 to 17% among the component populations (Table 2). In contrast, the values of P_K were always significantly greater (range: 37–68%) than compared to the P_E values within the same component population. In fact, there was no overlap with 95% CI (Figure 2). Thus, there is clear evidence for parasite cosibling transmission, and there is the potential for kin-mating to result in biparental inbreeding. Neither the interaction ($F_{4,73} = 1.0$) nor the main effects (population $F_{4,73} = 1.9$; intensity $F_{1,73} = 1.8$) were significantly associated to P_K per host based on the permutation-based ANCOVA (permutation p-values >.1). Population ($F_{4,77} = 1.5$) and intensity ($F_{1,77} = 0.6$) remained nonsignificant (permutation p-values >.1) after removing the interaction. As P_K per hosts had no relationship to infection intensities, the potential for biparental inbreeding would not be associated with infection intensities.

Among the five component populations, average within-host pairwise kinship coefficients were 0.32, 0.28, 0.22, 0.55 and 0.30, and 0.22, 0.27, 0.19, 0.47 and 0.20 for the Loiselle and Ritland estimators, respectively, for the five populations in order. Randomization tests indicated that parasite within-host kinship was significantly higher than expected by chance alone in each of the five component populations (p-values <.0001), thus indicating nonrandom transmission among hosts. In none of the five component populations was there a significant relationship between the individual host infection intensities and the average within-host–parasite kinship ($F_{1,14} = 2.5$, $p = .1$; $F_{1,20} = 0.1$, $p = .7$; $F_{1,12} = 0.9$, $p = .4$; $F_{1,8} = 0.1$, $p = .8$; $F_{1,19} = 0.1$, $p = .7$ based on Loiselle measures in component populations 1–5, respectively, and $F_{1,14} = 1.1$, $p = .3$; $F_{1,20} = 0.1$, $p = .8$; $F_{1,12} = 2.1$, $p = .2$; $F_{1,8} = 0.4$, $p = .6$; $F_{1,19} = 0.1$, $p = .8$ based on Ritland measures). These results indicated that infection intensity was not related to the potential for biparental inbreeding. Overall, the sibship reconstruction data were congruent with the analyses based on the Ritland and Loiselle kinship estimators in that related parasites were transmitted together more often than expected by chance, but that the percentage of possible outcrosses that could result in kin-mating was not related to the infection intensity.

3.5 | Estimating potential kin-mating rates and accounting for levels of inbreeding

When inbreeding was estimated from COLONY-selfing rates alone, four of the five estimated F_e values were significantly lower (at either $p < .05$ or $p < .01$) than the component population F_{IS} values (Figure 3). Thus, selfing rates alone cannot account for the observed levels of inbreeding within the component populations (exact binomial probability of four rejections of five tests under a null of no difference and $\alpha = 0.05$ is $p = .00003$). However, when incorporating kin-mating rates along with COLONY-selfing rates, none of the five estimated F_e values were significantly different from the component population F_{IS} values ($p > .05$, Figure 3). Taken collectively, the observed component population F_{IS} values can be accounted for by including both the COLONY-selfing rates and potential kin-mating rates. Thus, there is support for a significant contribution of biparental inbreeding to F_{IS} in each component population. Overall, kin-mating rates (t_K) were statistically the same among the locations and

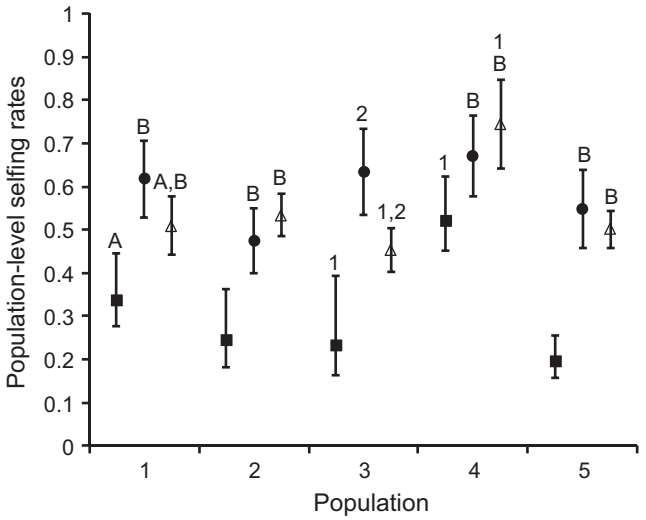

FIGURE 1 Comparisons of the realized population-level selfing rates to the potential population-level selfing rates. The realized rates are based on the pedigree reconstruction analysis, and the potential rates are based on the relationship of infection intensities to individual selfing rates from Detwiler et al. (2017). Solid squares and solid circles represent the potential estimates based on the assumptions of random reproductive success (NC) and density-dependent fecundity (C), respectively. Within each population, these values are compared to the COLONY-selfing rates, open triangles. Shown are 84% CI. Letters denote 84% CI overlap (i.e., estimates are not statistically different with a $p > .05$) between the COLONY-selfing rate and the NC-estimate (A) or C-estimate (B). Numbers denote there is overlap at the 94% CI (i.e., estimates are not statistically different with a $p > .01$) between the COLONY-selfing rate and the NC-estimate (1) or C-estimate (2)

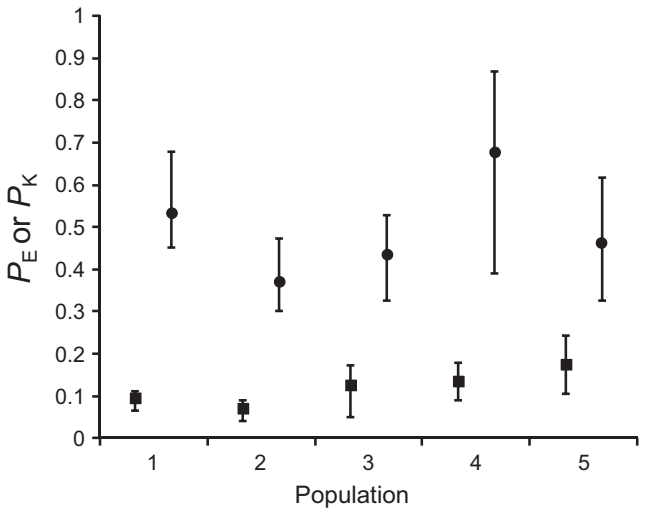

FIGURE 2 Percentage of kin dyads in the each component population as a whole (P_E, solid squares) and within hosts (P_K, circles). 95% CI are shown

ranged from 17% to 26% (Table 2, Figure 4). Only the pairwise comparison of Population 1 to Population 2 did not overlap with 84% CI, but it did at the 94% CI.

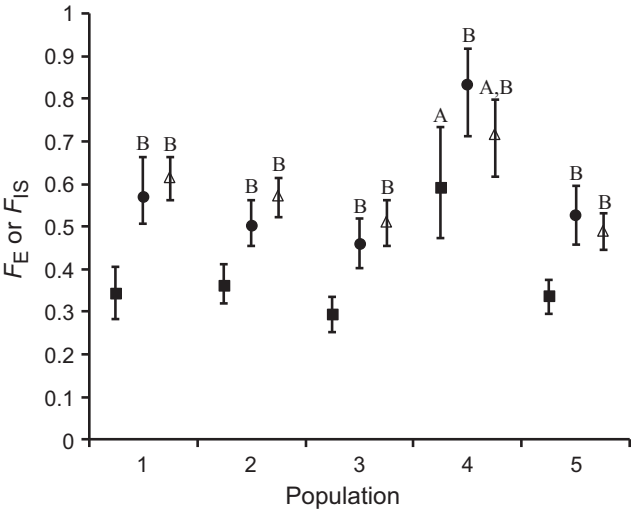

FIGURE 3 Comparisons of F_{IS} (open triangles) to F_e based on COLONY-selfing rates alone (filled squares) and to F_e based on COLONY selfing rates and potential kin-mating rates (filled circles). 84% CI are shown. Letters denote 84% CI overlap (i.e., estimates are not statistically different with a $p > .05$) between F_{IS} and F_e based on COLONY-selfing rates alone (A) or F_e based on COLONY-selfing rates and potential kin-mating rates (B). The remaining four comparisons between F_e based on COLONY-selfing rates alone and F_{IS} did not overlap with a 94% CI (i.e., $p < .01$)

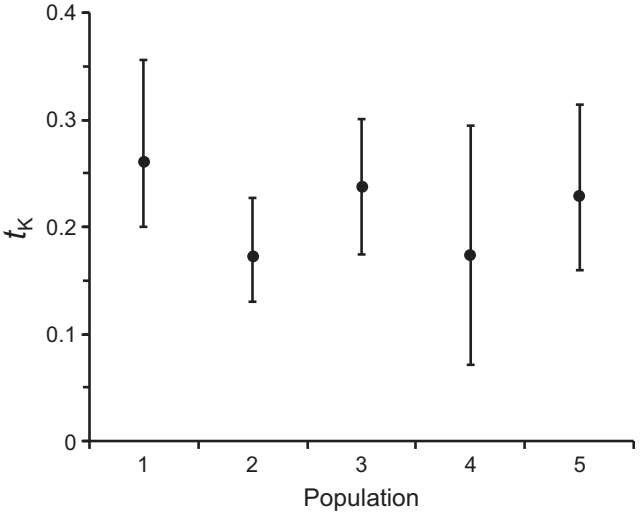

FIGURE 4 Overall potential kin-mating rates ($t_K = S_2 + S_3$). 95% CI are shown.

4 | DISCUSSION

4.1 | Characterization of overall genetic diversity and inbreeding

The overall patterns of low genetic diversity, high LD and high F_{IS} values (Table 1) for these component populations of *O. javaensis* are characteristic of a species with extensive nonrandom mating due to self-mating and/or kin-mating (Charlesworth, 2003; Jarne, 1995; Siol,

Prosperi, Bonnin, & Ronfort, 2008). While inbreeding from nonrandom mating alone does not result in the loss of gene diversity or cause LD in an infinite population (Templeton, 2006), when coupled with finite population dynamics, these genetic patterns emerge (e.g., Siol et al., 2008). For example, self-compatible hermaphroditic species have the capability of founding a new population with a single individual. If only one or a few individuals colonize a new location, there will be loss of gene diversity and LD will be generated via genetic drift from a founding event (Hedrick, 2011; Siol et al., 2008). Low gene diversity and LD will be retained for greater periods in a nonrandomly mating species relative to a randomly mating species because the effective population size of an inbred species will be lower (up to a half that of a randomly mating monoecious species) and the rate back to linkage equilibrium will be greatly retarded due to a reduced effective recombination rate (Nordborg, 2000; Pollak, 1987). Given that both *O. javaensis* and its Mediterranean gecko host are exotic species in the southern USA, along with the tapeworm's likely dependence on its host for broad geographic dispersal, founder events are expected to be a common population history feature for this tapeworm among the locations we sampled. The results are consistent with this plausible demographic history. Below we discuss the details of the parasite's high rates of nonrandom mating, which as mentioned above, will perpetuate the low diversity and LD resulting from such probable founder events.

4.2 | Realized selfing rates from pedigree reconstruction analyses

Comparisons of selfing rates from different life history stages can provide insight into the ecological and/or selective factors that shape an organism's mating system and population levels of inbreeding in general. Of particular interest, the odds ratio of selfed individuals between two life history stages can be used to infer selection for or against inbred individuals (Manly, 1985) where the relative fitness of selfed individuals is $w = (t_1 s_2 / s_1 t_2)$; s_i and t_i are the selfing and outcrossing rates, respectively, at a life history stage (Ritland, 1990). Inbreeding depression ($\delta = 1 - w$) would be inferred if the odds of a selfed individual were lower in the later life history stage whereas outbreeding depression (resulting in a negative δ) would be inferred for the reverse pattern.

Here, we provide a post hoc analysis of selection to illustrate a utility of pedigree reconstruction data, which can provide an estimate of the realized selfing rate at the sampled life history stage. In the current study, this is the proportion of selfed individuals that survived to adulthood (tapeworms collected from geckos). We used the COLONY-selfing rates for s_2 ($t_2 = 1 - s_2$). In Detwiler et al. (2017), we extrapolated the primary mating system to estimate potential population-level selfing rates that would be manifested at the metacestode stage (the juvenile stage that infects the intermediate host) by making assumptions about random reproductive success (NC-estimates) or density-dependent fecundity (C-estimates). Thus, we view the NC- and C-selfing rate estimates at the metacestode stage as plausible hypotheses to be tested (Detwiler et al., 2017). We used the potential selfing rate estimates from the metacestode stage for s_1 ($t_1 = 1 - s_1$).

In comparing the NC-selfing rates to the COLONY-selfing rates, four of five comparisons showed a significantly higher ($p < .05$) realized selfing rate (Figure 1). To explain this pattern, high outbreeding depression within these component populations would need to be invoked (mean among-population $\delta = -2.04$ is significantly <0, $t_4 = -5.41$, $p = .006$; Table 3). In contrast, when comparing the C-selfing rates to the realized rates, only one of five comparisons was different ($.01 < p < .05$, where the C-estimate was higher than the COLONY-estimate in Population 3; Figure 1). There is no overall evidence of selection on selfed individuals when metacestode selfing rates are based on the C-estimates (mean among-population $\delta = 0.07$ is not different than 0, $t_4 = 0.40$, $p = .71$, Table 3). Outbreeding depression has been found on local scales (Escobar, Nicot, & David, 2008; Grindeland, 2008). However, we view outbreeding depression at the scale of our component population samples as unlikely because it is more likely an among-population rather than within-population phenomena requiring hybridization events between diverged populations to disrupt local adaptive loci or coadapted gene complexes (Dolgin, Charlesworth, Baird, & Cutter, 2007; Schierup & Christiansen, 1996; Sletvold, Grindeland, Zu, & Agren, 2012). Moreover, there is a large body of support that density-dependent growth and fecundity operate across many helminth taxa, especially tapeworms (Poulin, 2007). Indeed, reduced body size at higher infection intensities has been reported in *O. javaensis* and another species in the genus, *O. bivitellobata* (Brooks & Mayes, 1976; Criscione & Font, 2001a). The NC- and C-estimates likely represent ends of a continuum along which crowding effects may impact realized population-level selfing rates. All of the COLONY-selfing rates were higher than the NC-estimates, but were sometimes between the NC- and C-estimates. Thus, density-dependent fecundity may not be as extreme as assumed in the C-estimates. Nonetheless and taken collectively over these patterns, we conclude that the realized selfing rates are consistent with hypothesis that crowding results in greater reproductive success among tapeworms that have higher selfing rates, that is, tapeworms from low-intensity infections, and thus boosts selfing rates beyond that expected from random reproductive success (Detwiler et al., 2017).

We acknowledge a more definitive assessment of the hypothesized NC- and C-estimates could come from pedigree reconstruction selfing rates estimated from a sample of metacestodes from nature. Unfortunately, we do not know the intermediate host in nature. Nevertheless, the purpose of the above post hoc analyses was to demonstrate how pedigree reconstruction data provide a useful means to generate comparisons of selfing rates from different life history stages of an organism. Such comparisons could greatly facilitate field-based inbreeding depression studies in any self-compatible hermaphroditic organism where different life stages can be collected. Currently, inbreeding depression studies on animal hermaphrodites are largely restricted to laboratory experiments where only "apparent inbreeding depression" (outcrossing cannot be enforced in selfing animals as it can in plants) can be estimated (Escobar et al., 2011). In

TABLE 3 Relative fitness of selfed individuals (w) and measure of selection on inbred individuals (δ) when comparing the realized selfing rates obtained from pedigree reconstruction data on an adult sample to extrapolated selfing-rate estimates (NC and C) based on parentage data at the metacestode stage (see main text for details). NC-estimates are based on the assumption of random reproductive success, and C-estimates are based on the assumption of density-dependent fecundity

	NC-estimates		C-estimates	
Population	w	δ	w	δ
1	2.05	−1.05	0.64	0.36
2	3.53	−2.53	1.26	−0.26
3	2.74	−1.74	0.48	0.52
4	2.66	−1.66	1.42	−0.42
5	4.21	−3.21	0.84	0.16

addition, "glasshouse" experiments often underestimate inbreeding depression compared to field-based estimates because such experiments do not account for "severe episodes of selection" that may occur in nature (Eckert & Barrett, 1994). Among parasitic flatworms, there are few assessments of inbreeding depression due to inherent difficulties in conducting experiments that involve the maintenance of a multihost life cycle. In some experimental studies, inbreeding depression has been found (Milinski, 2006) whereas others show mixed or no evidence of inbreeding depression (e.g., Lagrue & Poulin, 2009; Nollen, 1971; Rieger, Haase, Reusch, & Kalbe, 2013). We envision that comparisons of pedigree reconstruction selfing rates between parasite stages in intermediate hosts and final hosts will open the door to exploring the role of inbreeding depression in parasitic flatworm evolution.

4.3 | Assessing cosibling transmission and subsequent potential for kin-mating

Analyses based on both the average within-host kinship estimators (Ritland and Loiselle) and pedigree reconstruction data provided evidence that related parasites were cotransmitted. Taken at face value (but see Wang, 2014), the within-host–parasite kinship values are on the order of half- to full-siblings (i.e., 0.125 and 0.25, respectively). The sibship reconstruction data confirmed that full- and half-sibs were being transmitted together (Table 2). In addition, the percentages of kin dyads were greater within hosts than expected given the frequencies of kin dyads across each of the respective component populations (Figure 2). Because kin dyads occur at much higher frequencies within hosts compared to the component population as a whole, there was empirical support for potential kin-mating in influencing the levels of F_{IS} within each population.

In contrast to the inverse power relationship observed between infection intensities and selfing rates (Detwiler et al., 2017), no significant relationships between the average kinship of parasites within hosts or P_K per host relative to the infection intensities were found. Thus, potential kin-mating rates are not associated with infection intensities. This result has implications for modelling how parasite sibling transmission impacts component population inbreeding. For example, Dharmarajan (2015) modelled cosibling transmission for a dioecious parasite as a negative binomial to mimic commonly observed aggregated distributions found among metazoan parasites (Shaw & Dobson, 1995; Shaw, Grenfell, & Dobson, 1998). The "aggregation" parameter of Dharmarajan (2015) itself is a function of the number and size of sib groups and thus, possibly reflects cosibling transmission that may not be independent of the distribution of infection intensities themselves (as suggested by the results in Figure 2 of the study where biparental inbreeding decreases with higher mean infection intensities). If the model of Dharmarajan (2015) imposes a codependence between cosibling transmission and infection intensities, our empirical results contrast with this model. At least for *O. javaensis*, the potential for biparental inbreeding as measured by P_K is decoupled from the distribution of infection intensities.

4.4 | Estimating potential kin-mating rates and accounting for levels of inbreeding

Partitioning the mating system components into self-mating and kin-mating can provide insight into the ecological factors that contribute to inbreeding (Herlihy & Eckert, 2004; Williams, 2007) and is critical for understanding the evolution of the mating system itself (Porcher & Lande, 2016; Ronfort & Couvet, 1995). Unfortunately, there are no estimates of kin-mating rates among flatworm parasites, and therefore, the potential for biparental inbreeding has only been assessed indirectly via analyses of nonrandom transmission among hosts (reviewed in Gorton et al., 2012). In part, this is because there are few methods that can be applied to flatworm parasites to disentangle selfing and kin-mating rates. The most common method used to determine kin-mating rates is the difference in multilocus to single locus estimates of outcrossing from progeny-array data (Ritland, 2002). However, the progeny-array method can underestimate this difference with few markers (Griffin & Eckert, 2003; Ritland, 2002). Although using more markers may improve the estimate, in practice, collection of progeny-array data from nature is difficult in many flatworm parasite systems. Alternative methods to determine kin-mating rates are few and include experimental transplants and floral emasculation among hermaphroditic plants (Griffin & Eckert, 2003; Herlihy & Eckert, 2004). Such methods are impracticable or impossible in flatworm parasites. An alternative means of determining kin-mating contributions to inbreeding is to estimate the average pairwise kinship of spatially proximate individuals (Vekemans & Hardy, 2004). A kinship coefficient between potential mates provides a measure of potential biparental inbreeding as it is the expected inbreeding coefficient of their possible offspring. In conjunction with an overall measure of inbreeding in the population, Vekemans and Hardy (2004) used the measure of biparental inbreeding to estimate selfing rates. However, this method does not provide an independent measure of the selfing rate. Moreover, it is not clear how comparable estimates will be between populations or species as Wang (2014) notes that the magnitudes of pairwise kinship estimators are "more or less arbitrary, depending on the reference allele frequencies."

Pedigree reconstruction data along with the methods we employed in this study provide a feasible means of teasing apart selfing and kin-mating rates in natural flatworm parasite systems or more generally, organisms with closed mating systems. One simply needs to generate genotype data from a sample of adults and have knowledge of how individuals are partitioned into realistic mating groups (e.g., individual hosts in the case of endoparasites). Moreover, as the sibling reconstructions are performed without regard to which hosts individual parasites infected, the percentage of kin dyads within hosts (P_K, which is an estimate of $t_R/(t_R + t_U)$) is independently estimated from the realized selfing rate that is generated along with the reconstructed pedigree. Nonetheless, our conversion of the percentage of kin dyads within hosts to a kin-mating rate represents a "potential" rate that contributes to biparental inbreeding, analogous to the method of Vekemans and Hardy (2004). As a result, we suggest three factors to consider prior to calculating a potential kin-mating rate.

First, a test of whether the frequency of related dyads within hosts is greater than expected by chance is needed to determine whether kin-mating will impact F_{IS}. As discussed above, we had strong evidence for this in each of the component populations (Figure 2). Second, an assumption about how outcrossing among unrelated and kin dyads occurs within hosts needs to be made. We made the assumption that it occurred randomly, and our overall assessment of the factors that contribute to F_{IS} suggests this was a reasonable assumption. If there was a preference for mating with unrelated individuals, realized kin-mating rates would be lower and vice versa if there was preference for related individuals. We do note that mate choice based on relationship status may be possible in flatworm parasites. A study with the tapeworm *Schistocephalus solidus* (a distantly related species with a very different life cycle than our study species) indicated that related individuals spent more time together in a paired-choice experiment with an unrelated individual, although actual mating rates were not assessed via parentage analysis (Schjørring & Jäger, 2007). If one had evidence of mate preference based on relatedness, it should be possible to adjust the calculation of P_K accordingly. Third, we recommend testing whether P_K per host (or average within-host relatedness) is associated with infection intensities. In our system, we found no evidence of a relationship. However, if an association is found (positive or negative), the weights in the calculation of P_K could be changed to $1/H_{i>1}$ where $H_{i>1}$ is the number of hosts within an intensity >1. This would enable estimates under the assumption of density-dependent fecundity (see Detwiler et al., 2017).

The overall potential kin-mating rates we observed were high (mean among the five component populations = 21.5%; range: 17%–26%; Table 2, Figure 4) relative to what has been described for hermaphroditic plants. In a comprehensive survey of kin-mating rates estimated from 276 hermaphroditic plants (all based on the progeny-array approach), Porcher and Lande (2016) reported a low mean kin-mating rate of 3.3% where <10% of the species had values >10% and a max rate of 21.8%. Progeny-array estimates from hermaphroditic animals are similarly low (Table S6). Consistent with the possibility that results based on progeny-array data are underestimates, experimental manipulations with plants have yielded estimates of kin-mating almost 10 times greater than compared to progeny-array analyses (mean of two transplant populations = 27.8% compared to 2.9% from progeny-array estimates, Griffin & Eckert, 2003; mean of 6 emasculated populations = 13.8% compared to 1.4% from progeny-array estimates, Herlihy & Eckert, 2004). The kin-mating rates in these latter two studies are comparable with levels of kin-mating observed in our system.

Our methods provide potential kin-mating rates for which it might be argued do not accurately reflect realized kin-mating rates. However, the realized selfing rates alone cannot account for all the inbreeding (measured as F_{IS}) in the component populations (Figure 3). Therefore, it is logical to conclude that a component of F_{IS} is due to biparental inbreeding. Indeed, when incorporating potential kin-mating rates (S_2 and S_3) along with COLONY-selfing rates, the F_{IS} in all five component populations can be explained by the estimated F_e at $\alpha = 0.05$ (Figure 3). In addition, there was a significant positive correlation between the F_e based on COLONY-selfing rates and potential kin-mating rates and F_{IS} (Pearson $r = .9$, $p = .04$). Given these results, we infer that the potential kin-mating rates are a reasonable reflection of the realized kin-mating rates in this system. Taken collectively, these results also suggest the sampled populations are in inbreeding equilibrium. Whether the higher kin-mating rates we observed represent a greater propensity for biparental inbreeding in parasites with lifestyles that promote cosibling transmission or whether most prior estimates in other species are just an artefact of progeny-array underestimates remains to be determined.

4.5 | Summary

We provided an in-depth analysis of the local population genetic structure of the tapeworm *O. javaensis*. This parasite shows all the classic signs of an inbred organism that is subject to founder events, that is, low genetic diversity, high F_{IS} values and high LD. What sets our study apart, however, is that we discovered a link between the transmission process and inbreeding. In Detwiler et al. (2017), we showed how individual tapeworm selfing rates were a function of infection intensities, an emergent property of the transmission process. In this study, we found when using the distributions of parasites among hosts along with the assumption of density-dependent fecundity that extrapolated population-level estimates could explain the realized selfing rates obtained from pedigree reconstruction analyses. However, the realized selfing rates alone could not account for all of the inbreeding within populations. We used a series of unique analyses that capitalized on pedigree reconstruction data and the closed mating systems of parasites to show that sibling parasites are cotransmitted at a greater frequency than expected by chance alone. Moreover, we provided a novel means of assessing the role of kin-mating in influencing inbreeding within parasite populations. Incorporating estimates of potential kin-mating rates based on the percentage of kin dyads within hosts along with selfing-rate estimates, we could account for the observed levels of inbreeding within the component populations. This latter result suggests that the sampled populations are in

inbreeding equilibrium. Overall, our results indicate that the transmission process in terms of the number of and relatedness of cotransmitted parasites can impact parasite inbreeding. The methods we employed should be broadly applicable to other flatworm parasites or more generally species with closed mating systems. As such, we foresee that a new frontier will emerge wherein the diverse life histories of flatworm parasites could be utilized in comparative evolutionary studies to broadly address ecological factors or life history traits that drive mating systems and hence inbreeding in natural populations.

ACKNOWLEDGEMENTS

We thank the following undergraduates for help with field sampling, gecko and beetle dissections, and/or molecular laboratory work: Katherine Holley, Erika Medina (Louis Stokes Alliance for Minority Participation researcher), Kathleen Peart, Kwadwo Owusu, Moinuddin Syed, Brianna Trejo and Abbie Ince. Several people helped with gecko collections including Emily Kasl, Mary Janecka and Matthew Gasner. We also thank Adam Jones and Isabel Caballero for discussions pertaining to the study and Jinliang Wang for his helpful and rapid responses to our inquiries regarding COLONY. CDC is supported by a National Science Foundation grant (DEB1145508).

DATA ACCESSIBILITY

The genotype data used in the analyses are included as Table S4.

AUTHOR CONTRIBUTION

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. generated the genetic data. C.D.C. developed the methods for estimating the kin-mating rates. J.T.D. and C.D.C. performed analyses and wrote the manuscript.

REFERENCES

Anderson, T. J. C., Romero-Abal, M. E., & Jaenike, J. (1995). Mitochondrial DNA and *Ascaris* microepidemiology: The composition of parasite populations from individual hosts, families and villages. *Parasitology*, *110*, 221–229.

Anthes, N., Putz, A., & Michiels, N. K. (2006). Sex role preferences, gender conflict and sperm trading in simultaneous hermaphrodites: A new framework. *Animal Behaviour*, *72*, 1–12.

Belkhir, K., Borsa, P., Chikhi, L., Rausfast, N., & Bonhomme, F. (2004). GENETIX 4.05, Windows TM Software for Population Genetics. Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Universite′ Montpellier II, Montpellier, France.

Brooks, D. R., & Mayes, M. A. (1976). Morphological variation in natural infections of *Oochoristica bivitellobata* Loewen, 1940 (Cestoidea: Anoplocephalidae). *Transactions of the Nebraska Academy of Sciences*, *3*, 20–21.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology*, *83*, 575–583.

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society, Series B*, *358*, 1051–1070.

Cornell, S. J., Isham, V. S., Smith, G., & Grenfell, B. T. (2003). Spatial parasite transmission, drug resistance, and the spread of rare genes. *Proceedings of the National Academy of Sciences*, *100*, 7401–7405.

Criscione, C. D., & Blouin, M. S. (2005). Effective sizes of macroparasite populations: A conceptual model. *Trends in Parasitology*, *21*, 212–217.

Criscione, C. D., & Font, W. F. (2001a). Development and specificity of *Oochoristica javaensis* (Eucestoda: Cyclophyllidea: Anoplocephalidae: Linstowiinae). *Comparative Parasitology*, *68*, 149–155.

Criscione, C. D., & Font, W. F. (2001b). Artifactual and natural variation of *Oochoristica javaensis*: Statistical evaluation of in situ fixation. *Comparative Parasitology*, *68*, 156–163.

Criscione, C. D., & Font, W. F. (2001c). The guest playing host: Colonization of the introduced Mediterranean gecko, *Hemidactylus turcicus*, by helminth parasites in southeastern Louisiana. *Journal of Parasitology*, *87*, 1273–1278.

Criscione, C. D., Poulin, R., & Blouin, M. S. (2005). Molecular ecology of parasites: Elucidating ecological and microevolutionary processes. *Molecular Ecology*, *14*, 2247–2257.

Detwiler, J. T., Caballero, I. C., & Criscione, C. D. (2017). Role of parasite transmission in promoting inbreeding: I. Infection intensities drive individual parasite selfing rates. *Molecular Ecology*, https://doi.org/10.1111/mec.14211

Detwiler, J. T., & Criscione, C. D. (2011). Testing Mendelian inheritance from field-collected parasites: Revealing duplicated loci enables correct inference of reproductive mode and mating system. *International Journal for Parasitology*, *41*, 1185–1195.

Detwiler, J. T., & Criscione, C. D. (2014). Recently introduced invasive geckos quickly reach population genetic equilibrium dynamics. *Biological Invasions*, *16*, 2653–2667.

Dharmarajan, G. (2015). Inbreeding in stochastic subdivided mating systems: The genetic consequences of host spatial structure, aggregated transmission dynamics and life history characteristics in parasite populations. *Journal of Genetics*, *94*, 43–53.

Dolgin, E. S., Charlesworth, B., Baird, S. E., & Cutter, A. D. (2007). Inbreeding and outbreeding depression in *Caenorhabditis* nematodes. *Evolution*, *61*, 1339–1352.

Eckert, C. G., & Barrett, S. C. H. (1994). Inbreeding depression in partially self-fertilizing *Decodon verticillatus* (Lythraceae): Population-genetic and experimental analyses. *Evolution*, *48*, 952–964.

Escobar, J. S., Auld, J. R., Correa, A. C., Alonso, J. M., Bony, Y. K., Coutellec, M. A., . . . David, P. (2011). Patterns of mating-system evolution in hermaphroditic animals: Correlations among selfing rate, inbreeding depression, and the timing of reproduction. *Evolution*, *65*, 1233–1253.

Escobar, J. S., Nicot, A., & David, P. (2008). The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics*, *180*, 1593–1608.

Gao, H., Williamson, S., & Bustamante, C. D. (2007). A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics*, *176*, 1635–1651.

Gorton, M. J., Kasl, E. L., Detwiler, J. T., & Criscione, C. D. (2012). Testing local-scale panmixia provides insights into the cryptic ecology, evolution, and epidemiology of metazoan animal parasites. *Parasitology*, *139*, 981–997.

Goudet, J. (1995). FSTAT v1.2: A computer program to calculate *F*-statistics. *Journal of Heredity*, *86*, 485–486.

Griffin, C. A. M., & Eckert, C. G. (2003). Experimental analysis of biparental inbreeding in a self-fertilizing plant. *Evolution*, *57*, 1513–1519.

Grindeland, J. M. (2008). Inbreeding depression and outbreeding depression in *Digitalis purpurea*: Optimal outcrossing distance in a tetraploid. *Journal of Evolutionary Biology*, *21*, 716–726.

Hardy, O., & Vekemans, X. (2002). SPAGeDI: A versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, *2*, 618–620.

Hedrick, P. W. (2011). *Genetics of populations*, 4th edn. Sudbury, MA: Jones and Bartlett Publishers.

Hedrick, P. W., & Cockerham, C. C. (1986). Partial inbreeding: Equilibrium heterozygosity and the heterozygosity paradox. *Evolution*, *40*, 856–861.

Herlihy, C. R., & Eckert, C. G. (2004). Experimental dissection of inbreeding and its adaptive significance in a flowering plant, *Aquilegia canadensis* (Ranunculaceae). *Evolution*, *58*, 2693–2703.

Hood, G. M. (2011). PopTools v3.2.5. Retrieved from http://www.poptools.org

Jarne, P. (1995). Mating system, bottlenecks and genetic polymorphism in hermaphroditic animals. *Genetical Research*, *65*, 193–207.

Jarne, P., & Auld, J. R. (2006). Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals. *Evolution*, *60*, 1816–1824.

Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, *10*, 551–555.

Lagrue, C., & Poulin, R. (2009). Heritability and short-term effects of inbreeding in the progenetic trematode *Coitocaecum parvum*: Is there a need for the definitive host? *Parasitology*, *136*, 231–240.

MacGregor-Fors, I., & Payton, M. E. (2013). Contrasting diversity values: Statistical inferences based on overlapping confidence intervals. *PLoS ONE*, *8*, e56794.

Manly, B. F. J. (1985). *The statistics of natural selection*. New York, NY: Chapman and Hall.

de Meeûs, T., McCoy, K. D., Prugnolle, F., Chevillon, C., Durand, P., Hurtrez-Bousses, S., & Renaud, F. (2007). Population genetics and molecular epidemiology or how to "debusquer la bete". *Infection Genetics and Evolution*, *7*, 308–332.

Milinski, M. (2006). Fitness consequences of selfing and outcrossing in the cestode *Schistocephalus solidus*. *Integrative and Comparative Biology*, *46*, 373–380.

Nadler, S. A. (1995). Microevolution and the genetic structure of parasite populations. *Journal of Parasitology*, *81*, 395–403.

Nollen, P. M. (1971). Viability of a self-fertilizing strain of *Philophthalmus megalurus* (Trematoda: Digenea). *Journal of Parasitology*, *57*, 1222–1226.

Nordborg, M. (2000). Linkage disequilibrium, gene trees and selfing: An ancestral recombination graph with partial self-fertilization. *Genetics*, *154*, 923–929.

Pollak, E. (1987). On the theory of partially inbreeding finite populations. I. Partial selfing. *Genetics*, *117*, 353–360.

Porcher, E., & Lande, R. (2016). Inbreeding depression under mixed outcrossing, self-fertilization and sib-mating. *BMC Evolutionary Biology*, *16*, 105.

Poulin, R. (2007). *Evolutionary ecology of parasites*, 2nd edn. Princeton, NJ: Princeton University Press.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org/

Rieger, J. K., Haase, D., Reusch, T. B. H., & Kalbe, M. (2013). Genetic compatibilities, outcrossing rates and fitness consequences across life stages of the trematode *Diplostomum pseudospathaceum*. *International Journal for Parasitology*, *43*, 485–491.

Ritland, K. (1990). Inferences about inbreeding depression based on changes of the inbreeding coefficient. *Evolution*, *44*, 1230–1241.

Ritland, K. (2002). Extensions of models for the estimation of mating systems using *n* independent loci. *Heredity*, *8*, 221–228.

Ronfort, J., & Couvet, D. (1995). A stochastic model of selection on selfing rates in structured populations. *Genetical Research*, *65*, 209–222.

Rousset, F. (2008). GENEPOP ' 007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103–106.

Scharer, L. (2009). Tests of sex allocation theory in simultaneously hermaphroditic animals. *Evolution*, *63*, 1377–1405.

Schierup, M. H., & Christiansen, F. B. (1996). Inbreeding depression and outbreeding depression in plants. *Heredity*, *77*, 461–468.

Schjørring, S., & Jäger, I. (2007). Incestuous mate preference by a simultaneous hermaphrodite with strong inbreeding depression. *Evolution*, *61*, 423–430.

Selcer, K. W. (1986). Life history of a successful colonizer: The Mediterranean gecko, *Hemidactylus turcicus*, in southern Texas. *Copeia*, *1986*, 956–962.

Shaw, D. J., & Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: A quantitative review. *Parasitology*, *111*, S111–S133.

Shaw, D. J., Grenfell, B. T., & Dobson, A. P. (1998). Patterns of macroparasite aggregation in wildlife host populations. *Parasitology*, *117*, 597–610.

Siol, M., Prosperi, J. M., Bonnin, I., & Ronfort, J. (2008). How multilocus genotypic pattern helps to understand the history of selfing populations: A case study in *Medicago truncatula*. *Heredity*, *100*, 517–525.

Sletvold, N., Grindeland, J. M., Zu, P. J., & Agren, J. (2012). Strong inbreeding depression and local outbreeding depression in the rewarding orchid *Gymnadenia conopsea*. *Conservation Genetics*, *13*, 1305–1315.

Templeton, A. R. (2006). *Population genetics and microevolutionary theory*. Hoboken, NJ: John Wiley & Sons Inc.

Uyenoyama, M. K. (1986). Inbreeding and the cost of meiosis: The evolution of selfing in populations practicing biparental inbreeding. *Evolution*, *40*, 388–404.

Vekemans, X., & Hardy, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology*, *13*, 921–935.

Wang, J. (2013). A simulation module in the computer program colony for sibship and parentage analysis. *Molecular Ecology Resources*, *13*, 734–739.

Wang, J. (2014). Marker-based estimates of relatedness and inbreeding coefficients: An assessment of current methods. *Journal of Evolutionary Biology*, *27*, 518–530.

Wang, J. (2015). Does G_{ST} underestimate genetic differentiation from marker data? *Molecular Ecology*, *24*, 3546–3558.

Wang, J., El-Kassaby, Y. A., & Ritland, K. (2012). Estimating selfing rates from reconstructed pedigrees using multilocus genotype data. *Molecular Ecology*, *21*, 100–116.

Wang, J., & Santure, A. W. (2009). Parentage and sibship inference from multilocus genotype data under polygamy. *Genetics*, *181*, 1579–1594.

Waples, R. S. (2015). Testing for Hardy-Weinberg proportions: Have we lost the plot? *Journal of Heredity*, *106*, 1–19.

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.

Wheeler, B., & Torchiano, M. (2016). lmPerm: Permutation tests for linear models. R package version 2.1.0. Retrieved from http://CRAN.R-project.org/package=lmPerm

Williams, C. F. (2007). Effects of floral display size and biparental inbreeding on outcrossing rates in *Delphinium barbeyi* (Ranunculaceae). *American Journal of Botany*, *94*, 1696–1705.

SUPPORTING INFORMATION

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

How to cite this article: Detwiler JT, Criscione CD. Role of parasite transmission in promoting inbreeding: II. Pedigree reconstruction reveals sib-transmission and consequent kin-mating. *Mol Ecol*. 2017;26:4405–4417.
https://doi.org/10.1111/mec.14210