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Inbreeding and parasite sex ratios

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The breeding system of parasitic protozoa affects the evolution of drug resistance and virulence, and is relevant to disease diagnosis and the development of chemo- and immunotherapy. A major group of protozoan parasites, the phylum Apicomplexa, that includes the aetiological agents of malaria, toxoplasmosis and coccidiosis, all have dimorphic sexual stages. The sex ratio (proportion of males produced by parasites) is predicted to depend upon the inbreeding rate, and it has been suggested that sex-ratio data offer a relatively cheap and easy method for indirectly estimating inbreeding rates. Here, we exploit a new theoretical machinery to show that there are generally valid relationships between f, Wright's coefficient of inbreeding, and sex ratio, z^*, the generality being with respect to population structure. To focus the discussion, we concentrate on malaria and show that the previously derived result, $f = 1 - 2z^*$, does not depend on the artificial assumptions about population structure that were previously made. Not only does this justify the use of sex ratio as an indirect measure of f, but also we argue that it may actually be preferable to measure f by measuring sex ratios, rather than by measuring departures from Hardy–Weinberg genotypic proportions both in malaria and parasites more generally.

Keywords: malaria; gametocyte; plasmodium; apicomplexa; local mate competition

1. INTRODUCTION

Establishing the inbreeding rate of parasites is important for studies in clinical and epidemiological medicine as well as evolutionary biology (Tibayrenc *et al.* 1990; Hastings & Wedgewood-Oppenheim 1997). The type of breeding system of these parasites has proved hugely controversial, particularly in the context of *Plasmodium*, where inbreeding rates ranging from effective panmixia to full clonality have been proposed (reviewed by Walliker *et al.* (1998)). Much of the controversy concerns the inferences made from direct and indirect population and molecular genetic methods (Paul & Day 1998; Hey 1999). We have argued that sex-allocation theory provides an effective alternative method for estimating inbreeding rates (Read *et al.* 1992). Here, we show that this assertion is not dependent on the particular details of how these populations are structured, and argue that consequently the sex-ratio approach may be preferable to the more costly molecular approaches. The generality of the argument probably explains why the theory has successfully predicted inbreeding rates in malaria populations in Papua New Guinea (Read *et al.* 1992; Paul *et al.* 1995), sex ratios of *Leucocytozoon* populations in birds (Read *et al.* 1995) and *Toxoplasma* in humans (Sibley & Boothroyd 1992; West *et al.* 2000), as well as explaining broad patterns of sex-ratio variation across the entire Apicomplexa (Read *et al.* 2001; West *et al.* 2000, 2001*b*).

The following argument generalizes to any microparasites with dimorphic sexual stages, but for the sake of clarity, we use the terminology associated with malaria parasites. In the blood of the vertebrate host, clonal haploid lineages replicate asexually, circulating in the bloodstream for several hours (so they are well mixed) before attaching to capillary walls, after which transmission to a dipteran vector occurs via haploid sexual stages, the gametocytes. A single haploid lineage (clone) can give rise to both male and female gametocytes and controls the sex ratio. Within the gut of the dipteran host, female gametocytes give rise to a single female gamete, while male gametocytes release up to eight viable male gametes. Random mating occurs within 20 min of a blood meal; gametes from a single clonal lineage are self-compatible. Diploid zygotes are formed, which undergo meiosis to restore the haploid state. The haploid products of this meiosis, encysted with the oocyst, initiate the period of asexual replication, first in the mosquito and then back in a vertebrate host. A more detailed description can be found in Read *et al.* (1992).

Throughout, we take sex ratio as the proportion of gametocytes that are male. Due to the fact that matings occur among the parasite clones present in the few microlitres of blood taken up by the vector, local mate competition and selfing will occur if there are low numbers of clones present in a blood meal. In the limit when all gametes in a mating pool are contributed by a single clone, selection should favour female-biased sex ratios that maximize the number of zygotes that can be formed. Where gametes are contributed by many clones, sex ratios closer to 1 : 1 should be favoured because these will maximize the genetic representation of each clone in the zygote population. This argument is Hamilton's local mate competition model (Hamilton 1967) cast into a malaria context, and forms the basis for our claim that sex ratio can be used to infer population structure.

Read *et al.* (1992) first presented a model for malaria parasites in which n unrelated lineages coexist in an infection and showed that

$$f = 1 - 2z^*, \tag{1.1}$$

where f is the coefficient of inbreeding (e.g. Crow &

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Kimura 1970) and z^* is the evolutionarily stable sex ratio (they actually referred to 's', for 'selfing rate' instead of f). This was a rederivation of a classic result from evolutionary biology (Hamilton 1967) in the context of malaria. Subsequently, Dye & Godfray (1993) constructed a model for malaria parasites that incorporated the biologically realistic feature that zygotes contain the progenitors of two lineages, which may be related. To make further progress they assumed that infections are initiated by the progeny of exactly n independent, i.e. unrelated, zygotes resulting in $2n$ haploid lineages occurring in an individual infection.

However, this previous theoretical work has left obscure:

(i) the consequences for how f is estimated from sex-ratio data of zygote non-independence flowing from different types of population structuring that can arise by vector transmission (e.g. at the house, village or region level);
(ii) to what extent sex ratio can be used to measure inbreeding in parasites with different life histories.

Recent advances in the theoretical machinery used for analysing social evolution (Taylor & Frank 1996; Frank 1998) make it possible to derive equation (1.1) very easily without making restrictive assumptions, such as zygote independence. These techniques also make clear the centrality of the inbreeding coefficient f in determining sex ratios. This justifies the use of sex ratios to measure f.

First, we will derive equation (1.1) and then discuss its application.

2. THEORY

(a) *Basic model*

Largely, we follow the symbolism of Frank (1998). First, we derive the relationship between the evolutionarily stable strategy (ESS) sex ratio, z^*, and a quantity we will denote by f. We derive this relationship in the usual way by focusing on the optimal strategy of a focal individual lineage. In the first instance, f will enter the theory as the slope of the regression of average group sex ratio on the sex ratio played by the focal individual. We will then go on to see that this is, in fact, the inbreeding coefficient f.

Define y, the proportion of male gametocytes, as the sex ratio played by a focal individual lineage in a randomly chosen infection (so it is actually a random variable), and z, the average sex ratio played by the group in which that lineage is found, i.e. all the lineages in the infection that will contribute sexual stages to the vector, where mating takes place.

Fitness, $w(y, z)$, is proportional to

$$w(y,z) = 1 - y + \frac{y}{z}(1 - z). \tag{2.1}$$

This is the canonical model of local mate competition (Hamilton 1967; Taylor & Bulmer 1980). The fitness of the focal lineage is made up of two terms:

(i) the first term, $(1 - y)$, represents the number of female gametocytes that the focal lineage produces (functionally equivalent to daughters in classical sex-ratio theory);
(ii) the second term represents the proportion of the total number of female gametocytes that have their gametes fertilized by the male gametes of the focal lineage.

The ESS sex ratio, z^*, is given by the solution to

$$\left.\frac{\mathrm{d}w}{\mathrm{d}y}\right|_{y=z=z^*} = 0 \tag{2.2}$$

or

$$\frac{\partial w}{\partial y}\frac{\mathrm{d}y}{\mathrm{d}y} + \frac{\partial w}{\partial z}\frac{\mathrm{d}z}{\mathrm{d}y} = 0, \tag{2.3}$$

where it is understood that the derivatives are evaluated at $z = z^*$. (More precisely, the derivative $\mathrm{d}z/\mathrm{d}y$ is the derivative of the conditional expectation of the random variable z given that the random variable y takes a particular value.)

If we define

$$\frac{\mathrm{d}z}{\mathrm{d}y} = f, \tag{2.4}$$

then the solution to equation (2.3) is

$$z^* = \frac{1}{2}(1 - f), \tag{2.5}$$

which can be rearranged to yield equation (1.1).

The symbol f has entered our model as a stand-in for a derivative, $\mathrm{d}z/\mathrm{d}y$. We now interpret it to be the slope of the regression of group mean sex ratio on the sex ratio of a focal individual (see the more precise discussion after equation (2.3)). This substitution is one of the key elements of the modelling approach described in Taylor & Frank (1996) and Frank (1998) and is rigorously justified by the use of Price's theorem. In § 3, we will see that this regression slope is Wright's coefficient of inbreeding.

We cannot here recapitulate the theoretical results derived in the works of Taylor and Frank. But we will paraphrase Frank's attempt at an intuitive explanation of the substitutions made, which may be helpful to some readers (Frank 1998). The derivative, $\mathrm{d}z/\mathrm{d}y$, can be read as the slope of the relationship between group mean sex ratio, z, and the sex ratio played by the focal individual y. If an individual plays a higher sex ratio, then the average group sex ratio will also be higher, and it will be higher still if the other individuals in the group, as a result of relatedness, are also playing a higher sex ratio. Looking at the ensemble of groups from a statistical point of view, f is defined to be the slope of the regression of mean group sex ratio on the sex ratio of a focal individual.

We must emphasize that equation (2.5) may be invalid for genetics and life histories other than those we have described. The sex-ratio literature is very rich because there are many variations, especially when we enter the macroscopic world: (haplo)diploidy, differential dispersal of males and females, sex-biased competition for resources other than matings and so on. Our parasites are relatively simple: for example, females do not compete for 'food' (blood cells) because the 'feeding stages' are asexual; the correlation between competing male gametes and the correlation between uniting gametes are the same.

(b) f: *from regression slope to inbreeding coefficient*

To simplify further development, we will start by assuming that infections are initiated by the products of exactly n zygotes. These are not necessarily independent. There are exactly $2n$ lineages arising from n zygotes and the sex ratio played by the ith lineage in an infection is y_i. We will label our focal lineage 1 and, therefore, it plays sex ratio y_1. (Remember that y_1 is a random variable: our population is a population of infected organisms.) By definition, the slope of the regression of z on y_1, b_{zy_1}, is given by

$$f = b_{zy_1} = \frac{\mathrm{cov}(z,y_1)}{\mathrm{var}(y_1)}. \tag{2.6}$$

Since

$$z = \frac{y_1 + \ldots + y_{2n}}{2n}, \tag{2.7}$$

this expands as

$$f = b_{zy_1} = \frac{1}{2n}\left(\frac{\mathrm{cov}(y_1,y_1) + \mathrm{cov}(y_2,y_1) + \ldots + \mathrm{cov}(y_{2n},y_1)}{\mathrm{var}(y_1)}\right)$$
$$= \frac{1}{2n}(b_{y_1,y_1} + b_{y_2,y_1} + b_{y_3,y_1} + \ldots b_{y_{2n},y_1}). \tag{2.8}$$

So, f is the average regression slope of the sex ratios produced by the lineages in the group on the sex ratio produced by the focal lineage. Or, to put it another way, it is the slope of the regression of the sex ratio of a randomly picked lineage on the sex ratio of the focal lineage. We now need to interpret this in terms of inbreeding.

The discussion of the meaning of inbreeding is often in terms of 'probability of identity by descent' of the uniting gametes, or whichever pair of gametes is being considered; a view introduced by Malecot (1948). However, Wright (1969) expressed his preference for interpreting the inbreeding coefficient in terms of the correlation between the uniting gametes. One clear difference that arises is that Wright's view allows f to be negative (that would correspond to an excess of heterozygotes). As the labelling of the gametes is arbitrary, the regression slope is the same as the correlation coefficient (consider the denominator; the variance of gamete 'X' cannot be different to that of 'Y'). Wright (1969) and Crow & Kimura (1970) demonstrate the validity of this statistical view of inbreeding.

We will label the indices in the regression slopes of equation (2.8) as follows. As already noted, the focal lineage is 1. The lineage arising from the other lineage that formed the zygote is 2. We are now free to add other levels of non-randomness that may be of concern. For example, the lineages in the zygotes from the same mosquito are labelled 3 and 4. The lineages from the zygotes from a mosquito from the same village are 5 and 6, and so on.

The regression slopes in equation (2.8) are, successively, the inbreeding coefficient of the focal lineage with: (i) itself (slope = 1), (ii) its zygote mate, and (iii) a lineage from a zygote from the same mosquito and so on. Assuming that each of these lineages has an equal probability $1/2n$ of fertilizing (or being fertilized by) gametes from the focal lineage then the expression on the right-hand side of equation (2.8) is the inbreeding coefficient f.

Equation (2.8) makes it clear how different levels of population structure may contribute to determining the inbreeding coefficient without compromising the simple relationship between sex ratio and inbreeding, as in equation (1.1). In modelling inbreeding or sex-ratio questions, it is the actual calculation of f, i.e. the calculation of the various regression slopes in equation (1.1), that considerably complicates the analysis. One of the great advantages of the modelling approach taken here is that we do not need to calculate them in order to establish the relationship between f and sex ratio, z^*. Other versions of equation (2.8) have been previously presented and in § 3 we relate them all to each other, as well as generalizing the result to the more realistic situation in which the number of lineages in an infection, n, is not a constant.

One liberty we have taken above is the following. Strictly speaking, the above slopes should be regressions of sex-ratio phenotype on genic values. We have effectively ignored the distinction between genotype and phenotype. This is justified in the present context as long as we are happy to assume that the genetic system is such that optimal sex-ratio phenotypes can be produced. This is a common, and reasonable, assumption and is discussed extensively in Frank (1998).

A different approach that one could take in order to emphasize that equation (2.5) is not dependent on any particular form of population structuring is to observe that the expression $(1-f)$ can be decomposed for levels of population sub- (and sub-sub) structuring using the F-statistics of Wright (1969). Many will be familiar with the decomposition corresponding to the division of a population into one level of sub-populations: $(1-f) = (1-F_{\mathrm{IT}}) = (1-F_{\mathrm{IS}})(1-F_{\mathrm{ST}})$.

3. DISCUSSION

(a) *Using sex ratios to estimate inbreeding rates*

We have shown that the predicted relationship, $f = 1 - 2z^*$, between the inbreeding rate (f) and the sex ratio (z^*) is extremely robust and general. In particular, although the various possible levels at which the population may be structured will certainly influence f and z, such structuring does not influence the fundamental relationship between them.

Why does the original simple model work so well in the face of population structuring at various levels (e.g. human, house, village and region)? The original derivation of equation (1.1) by Read *et al.* (1992) followed from the classical model of Hamilton (1967) for local mate competition developed for insects. It might be thought that this simple model would not hold up to complicated forms of population structuring that may occur in parasite populations. Indeed, several more elaborate models have previously been developed to examine how population structuring through limited dispersal influences the sex ratio (e.g. Bulmer 1986; Courteau & Lessard 2000; Frank 1985, 1986; Taylor & Frank 1996). Our work shows that, although such population structuring does influence the optimal sex-ratio strategy, because it also influences the inbreeding rate, the relationship between them as predicted by equation (1.1) holds. (See Queller (1994) for an analogous situation for measures of relatedness and the evolution of altruism in structured populations.)

Equation (1.1) makes two important assumptions in

order to hold for malaria and other parasites. First, if there was a possibility that female gametes would not encounter male gametes then a less female-biased sex ratio is predicted as a form of 'fertility insurance'. West *et al.* (2001*c*) formalize the effect of this for sex-ratio evolution but conclude that, at least for most Apicomplexan parasites, this is unlikely to affect our ability to infer selfing rate from sex-ratio data. Here, we merely note that equation (1.1) must be interpreted as referring to the sex ratio produced at an infection stage when fertility insurance is not a significant problem for the parasite, which will be when most gametocytes are produced anyway. Second, equation (1.1) assumes that male gametes produce enough male gametes to fertilize the female gametes. This places a lower bound on the sex ratio of $1/(1 + c)$, where c is the average number of viable gametes released from a male gametocyte (Read *et al.* 1992). The parameter c ranges from 4 to 8 in malaria and related blood parasites (Read *et al.* 1992), and from 6 to 1000 in eimeriorin blood parasites (West *et al.* 2000).

Our results support the underlying logic of using sex-ratio data to estimate the inbreeding rate in parasites (Read *et al.* 1992; West *et al.* 2000). In particular, the intimate relationship between f and sex ratio is not model specific: f enters the theory in equation (1.1), which does not presuppose any particular population structure. As observed above, other models suited to parasites with different life histories may be required: such bespoke models can be constructed, and solved, in the same way. It is important to note that high levels of confidence in one's model are only important if one wishes to come up with a point estimate of f. In many cases, there is more interest in ranking different populations in terms of their level of inbreeding (e.g. Paul *et al.* 1995; West *et al.* 2001*a*). In this case, ranking can be done by sex-ratio bias (higher female bias − higher f).

We will now argue that it is in fact preferable to measure the inbreeding rate indirectly with the sex-ratio data. One reason is practical, arising from limitations in the current technology used for measuring f directly. The more important reason is theoretical: we will argue that, in principle, it is better to measure f by measuring the sex ratio.

The practical problem is that parasite samples contain an unknown number of clones at unknown frequencies. This raises two problems. First, the probability of detecting a clone by polymerase chain reaction is almost certainly affected by the concentration and ratios of competing template (Kyes *et al.* 1997), but precisely how is not well understood. Even if it was, there would be considerable statistical difficulties in correcting for biases when the number and frequency of clones present is unknown. Second, non-amplifying (null) allelles are a feature of this sort of analysis. If these represent undetected alleles, perhaps because of primer mismatch, estimates of inbreeding coefficients can be severely biased (Anderson *et al.* 2000). The extent to which these problems undermine current molecular estimates of inbreeding rates in malaria parasites is unknown.

Even if technical and statistical advances remove this practical objection to the direct measurement of f, there still remains a big problem. Recall that f is a correlation coefficient. Such coefficients are only defined with respect to a specific population (or scale of measurement). So, for example, Hill *et al.* (1995) defined f with respect to a particular Tanzanian village from which the malaria data had come. But how do we know that this is the relevant population with respect to the broader, evolutionary issues that are, ultimately, of interest and not, for example, some larger region that includes the village? We do not. Furthermore, we do not have any way of discovering what this population is. But Mother Nature knows, and when she solves equation (1.1) to decide on the optimal sex ratio, She has calculated f over the evolutionarily relevant population. So the sex ratio played by the parasites in the Tanzanian village is calculated using the evolutionarily relevant population for calculating f. Notice that, as we argued above, this argument endorsing the indirect measure of f carries even more force if one is interested in ranking different populations in terms of their levels of inbreeding.

More generally, we are suggesting that a model-derived indirect measure of a biological quantity may be superior to direct measurement. Another example (and there are many others) comes from epidemiology: for pathogens that confer lifelong immunity, the measurement of the parasite's basic reproductive number cannot be achieved by the painstaking measurement of its component parameters but can be easily derived indirectly by simply measuring the average age at first infection.

(b) *Previous results*

Finally, we note that other versions of equation (2.5) have been previously presented and we will now relate them all to each other.

Hamilton (1979) presented, without derivation, the result

$$z^* = \frac{1}{2}\frac{V_{\mathrm{WG}}}{V_{\mathrm{T}}}, \tag{3.1}$$

where V_{WG} is the average within-group variance in sex ratio and V_{T} is the population variance.

Hamilton gave his notes to Frank who then provided a derivation of this result using Price's theorem (Frank 1986) and then replaced $V_{\mathrm{WG}}/V_{\mathrm{T}}$ with 'P', Wright's 'coefficient of panmixia'. The definition of P is, simply, $1 - f$. But, continuing in the Hamiltonian tradition, Frank did not offer any explanation for this substitution, perhaps because he felt it was too obvious. We will now justify it.

First, we will show that equation (3.1) is the same as equation (2.8). If the population variance, V_{T}, of a character, Y, is σ^2, then the expected variance of the character in a sample of size n, V_{WG}, is

$$V_{\mathrm{WG}} = \frac{n-1}{n}\sigma^2 - \frac{1}{n^2}\sum_{i\neq j}\mathrm{cov}(Y_i, Y_j). \tag{3.2}$$

The first term on the right-hand side is the familiar formula for independent variables Y. For the sake of familiarity, we will run our indices up to n, rather than the $2n$ that is actually appropriate here. So,

$$\frac{V_{\mathrm{WG}}}{V_{\mathrm{T}}} = \frac{n-1}{n} - \frac{1}{n^2\sigma^2}\sum_{i\neq j}\mathrm{cov}(Y_i, Y_j). \tag{3.3}$$

With f given by equation (2.8), we have

$$1 - f = \frac{n-1}{n} - \frac{1}{n\sigma^2}\sum_{i>1}\mathrm{cov}(Y_i, Y_1). \tag{3.4}$$

The apparent difference between these expressions arises from the fact that the former effectively allows each individual a turn at being the focal individual, which is just an arbitrary choice in the latter.

Using equation (3.3), we can see that

$$f = 1 - \frac{V_{WG}}{V_T} = \frac{\sum_{i,j} \mathrm{cov}(Y_i, Y_j)}{n^2\sigma^2}. \qquad (3.5)$$

This is the formula for the average correlation between the gametes of two randomly chosen lineages in the group, which is the correlational definition of Wright's f. Readers who are interested in the close relationship between a correlation and an analysis of variance—the latter implicated by the variance ratios—can consult the discussion in Fisher (1973) of the intraclass correlation coefficient. This average correlation coefficient is almost the same as the intraclass correlation coefficient, the difference being that this includes the terms $\mathrm{cov}(Y_i, Y_i)$ for all i.

One of us (A.F.R.) actually consulted Hamilton about optimal sex ratios in malaria in the very early 1990s and was handed three pages of notes deriving the following optimal sex ratio:

$$z^* = \frac{n-1}{2n}(1 - F). \qquad (3.6)$$

This was derived using an inclusive fitness approach, rather than the direct fitness approach adopted here, and assumed there are n lineages in an infection rather than $2n$. But this obviously does not account for the fact that n figures at all in the expression for optimal sex ratio (compare equation (2.5)), a feature that dissolves any potential use of the formula for the context discussed here. The discrepancy arises because, in our terms, Hamilton's F is the correlation between the sex ratio played by the focal lineage and a randomly chosen member of the group excluding the focal lineage, whereas the definition of f in equation (2.5) includes the focal lineage. This version of the optimal sex ratio languished in A.F.R.'s files until being disinterred for a meeting in honour of Hamilton after this paper was completed.

The generalization of the formula for f, equation (2.8), to a random number of zygotes is straightforward although somewhat cumbersome notationally. Suppose, for the sake of illustration, that $\mathrm{cov}(Y_i, Y_1) = 0$ for $i > 4$. Then

$$\begin{aligned} f &= \Pr\{N=1\}\frac{1}{2}(b_{Y_1,Y_1} + b_{Y_2,Y_1}) + \sum_{n=2}^{\infty} \Pr\{N=n\}\frac{1}{4} \\ &\quad \times (b_{Y_1,Y_1} + b_{Y_2,Y_1} + b_{Y_3,Y_1} + b_{Y_4,Y_1}) \\ &= \frac{1}{2h(n)}(b_{Y_1,Y_1} + b_{Y_2,Y_1} + b_{Y_3,Y_1} + b_{Y_4,Y_1}), \end{aligned} \qquad (3.7)$$

where $h(n)$ is the harmonic mean of n.

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