

Research article

Estimation of individual level of inbreeding using relatedness measures in haplodiploids

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Abstract. Although male haploidy in haplodiploid species aids purging of deleterious alleles, haplodiploid animals may nevertheless suffer significant negative effects of inbreeding. The effects may even be stronger in social Hymenoptera because the negative fitness consequences may be expressed at two levels: the individual level (inbred queens) and colony level (inbred workers). Surprisingly, in natural populations the impact of inbreeding on fitness has been studied in very few insects, and even fewer haplodiploid ones. Hence there is currently little understanding of the potential effects of inbreeding. One reason may be the difficulties in estimating inbreeding especially at the individual level, apart from the additional problems posed by haplodiploidy. In order to study the impact of inbreeding, its individual level must be estimated as precisely as possible. When the population pedigree is unknown, relatedness-based estimates of the individual inbreeding coefficient can be used to estimate inbreeding. Here we examine the relationship between inbreeding coefficients and relatedness in diploid and haplodiploid organisms, and provide guidelines for estimating inbreeding both at the individual and the colony level.

Keywords: Queen inbreeding, colony inbreeding, relatedness, haplodiploids.

The negative fitness consequences of inbreeding have been proposed to explain the evolution of species traits, such as sex biased dispersal or mate choice (Charlesworth and Charlesworth, 1987). In addition, inbreeding has been found to affect population extinction risk (e.g. Saccheri et al., 1998), thereby raising implications for conservation measures. Indeed, in many plants, mammals and birds, inbreeding may have strong

negative effects on individual fitness (Crnokrak and Roff, 1999; Keller and Waller, 2002). However, inbred populations do not necessarily suffer from inbreeding depression, and it is still unclear to what extent inbreeding may have shaped species biology. To better understand the potential impact of inbreeding on evolutionary changes, we need more comprehensive information on the fitness consequences of inbreeding, especially in natural populations. For example, the impact of inbreeding on fitness is poorly known in invertebrates (see however Saccheri et al., 1998; Haag et al., 2002), and among invertebrates, the effect of inbreeding in haplodiploids is particularly poorly known. Yet, about 20% of all animal species are haplodiploid (Evans et al., 2004) with males developing from unfertilised eggs and females from fertilised eggs. Among social Hymenoptera an increasing number of studies have shown that inbreeding is not such a rare phenomenon (e.g. Buschinger, 1989; Cole and Wiernasz, 1997; Keller and Fournier, 2002; Sundström et al., 2003; Stahlhut and Cowan, 2004). Hence, studies on the fitness consequences of inbreeding are needed. Interestingly, under male haploidy, inbreeding depression is expected to be relaxed compared to diploids because deleterious mutations are more easily purged (Werren, 1993). However, Werren (1993) also showed that some inbreeding depression should be observed in any case because not all genes are expressed in haploid males, and this has been confirmed empirically in a solitary Hymenoptera (Henter, 2003). More importantly, under single-locus complementary sex determination (i.e. when the gender of an individual is determined by a single locus as in the honey bee), the sex determining locus may play an important role in inbreeding depression (Beye et al., 2003). This is because individuals homozygous at this locus will develop into sterile males, rather than reproductive females or functional workers.

In natural populations the estimation of inbreeding may pose problems. If a pedigree of the study population is not available, neutral genetic marker loci can be used to obtain

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the individual genotypes at several loci. Based on the genotypes, the individual level of inbreeding can be estimated as the relatedness between maternal and paternal genes (Slate et al., 2004). Relatedness estimates obtained in this manner are more accurate than standardised heterozygosity estimates (Coltman et al., 1999), because relatedness estimates take into account the population-wide frequencies of each allele (Queller and Goodnight, 1989). The relationship between relatedness among parents and offspring inbreeding is largely known (Hartl and Clark, 1989). However, in haplodiploid organisms the relationship between inbreeding and relatedness is more complex, because, owing to haploidy, males cannot be inbred. Haplodiploidy also induces asymmetric relatedness between males and females: the relatedness of a male to a female always differs from the relatedness of a female to a male. Hence, in haplodiploid organisms the relationship between offspring inbreeding and relatedness among parents differs from that in diploid organisms. The relationship has been known for a long time (e.g. Hamilton, 1972), yet most researchers are not familiar with it. In order to provide simple guidelines for those studying social Hymenoptera, we first review the definition of relatedness, and then we report two methods that have been used to estimate individual inbreeding based on relatedness estimates. In each case, we will start by reviewing the diploid case before presenting the haplodiploid case.

Relatedness

Hamilton (1972) was the first to define regression relatedness when explaining his theory of kin-selection. He defined the relatedness of a focal altruist individual M to a receiver F as the ratio of the probability that M and F produce, for a given locus, two gametes identical by descent, and of the probability that M produces two gametes identical by descent for the same locus. It has been shown more recently that this definition can be translated into a ratio of coancestry (Lynch and Walsh, 1998; Milligan, 2003):

$$r_{MF} = \frac{\theta_{MF}}{\theta_{MM}} \quad (1),$$

with θ_{MF} being the coancestry between M and F, and θ_{MM} being the coancestry of M. By definition, the coancestry between M and F is equal to the coefficient of inbreeding in their (potential) offspring (F_O). If relatedness is estimated using the method developed by Queller and Goodnight (1989) then F_O is equal to the inbreeding definition of Wright (1984) (see Table 1 for summary of symbol definitions). Therefore, for both diploids and haplodiploids, the relatedness of an individual M to an individual F can be written as follows:

$$r_{MF} = \frac{F_O}{\theta_{MM}} \quad (2).$$

Given that the coancestry of M (θ_{MM}) is the probability that two gametes of M are identical by descent, θ_{MM} will depend on the ploidy of the focal individual (Table 2). Therefore, the relatedness in a haplodiploid organism is asymmetric

Table 1. Definition of symbols used in the manuscript

r_{MF}	Relatedness between the male and the female
r_{FM}	Relatedness between the female and the male
$\theta_{MF} = \theta_{FM}$	Coancestry between M and F (Probability that M and F produce identical gametes)
θ_{MM}	Coancestry of M (Probability that M produce two identical gametes)
F_O	Inbreeding coefficient of the offspring
F_M	Inbreeding coefficient of individual M

Table 2. Value of individual coancestry (θ_{MM}) for males and females in diploid and haplodiploid animals

	Males	Females
Diploids	$\frac{1}{2}(1 + F_M)$	$\frac{1}{2}(1 + F_F)$
Haplodiploids	1	$\frac{1}{2}(1 + F_F)$

because the coancestry of the focal individual (θ_{MM}) depends on its ploidy level. It is therefore important to choose precisely the focal individual M. For example, using the software Relatedness (Goodnight and Queller, 2001), the focal individual is defined under Px.

Internal relatedness

The first method to estimate the individual coefficient of inbreeding is to calculate individual internal relatedness (Amos et al., 2001), which is the relatedness between the two haploid genomes of an individual. All current estimates of relatedness assume no linkage among loci, so for a diploid animal the two alleles at each locus can be assumed to represent a random sample of the putative parental gametes, and the relatedness between the two gametes can be calculated straightforwardly. Given that the two putative parental gametes present in individual O (O1 and O2, respectively) can be regarded as haploid individuals, we can transform formula (1) and replace individual coancestry in accordance with Table 2 (case of haploid male):

$$r_{O1O2} = \frac{\theta_{O1O2}}{\theta_{O1O1}} = F_O \quad (3).$$

For haplodiploids, males are haploid so they cannot be inbred, but because females are diploid their internal relatedness can be calculated with the method used for diploid animals.

Relatedness among parents and offspring inbreeding in diploids

The second method to calculate the individual level of inbreeding (F_O) is based on the relatedness between its parents. The appropriate coefficient of coancestry (θ_{MM}) of the focal

parent (Table 2) is replaced in equation (1). The inbreeding coefficient for the offspring can be calculated from the relatedness of the male to the female. In this case, it also depends on the inbreeding coefficient of the male (Hamilton, 1972):

$$F_O = \frac{r_{MF}}{2}(1 + F_M) \quad (4).$$

Otherwise, the offspring inbreeding coefficient can be deduced from the relatedness of the female to the male, and in this case it also depends on the inbreeding coefficient of the female:

$$F_O = \frac{r_{FM}}{2}(1 + F_F) \quad (5).$$

The male or female inbreeding coefficients (F_M and F_F respectively) can be calculated from the internal relatedness as above. Finally, inbreeding can also be estimated based on the average relatedness between parents:

$$F_O = \frac{r_{MF}}{4}(1 + F_M) + \frac{r_{FM}}{4}(1 + F_F) \quad (6).$$

Using the average of the two relatedness estimates may improve the precision of the estimate, because it averages the error in each parents' individual inbreeding coefficient (F_M and F_F). However, the two estimates of relatedness are not independent so the error is multiplied by two. If neither of the parents is inbred, we find a well known formula (Hartl and Clark, 1989):

$$F_O = \frac{r_{FM}}{2} + \frac{r_{MF}}{2} \quad (6).$$

In such a situation, the relatedness of a male to a female is equal to the relatedness of a female to a male.

Relatedness among parents and offspring inbreeding in haplodiploids

In haplodiploids, the coefficient of inbreeding of the offspring can be similarly calculated from the relatedness between the parents. The differences arise because individual coancestry is different for haploid males and diploid females. If we estimate F_O from the relatedness of the female to the male we can use formula (5), because the individual coancestry for a female is the same as in diploid organisms. If F_O is estimated from the relatedness of the male to the female, the situation is much simpler because θ_{MM} is equal to one (Table 2). Therefore, the relatedness of the male to the female equals the offspring inbreeding coefficient:

$$F_O = r_{MF} \quad (8).$$

If inbreeding is estimated based on the average relatedness among parents, equation (5) and (8) can also be combined to give:

$$F_O = \frac{1}{2}r_{MF} + \frac{1}{4}r_{FM}(1 + F_F) \quad (9).$$

Discussion

In diploid organisms relatedness is increasingly often used to estimate inbreeding (e.g. Amos et al., 2001; Hansson, 2004; Hoffman et al., 2004). As suggested by Slate et al. (2004), relatedness is a better estimate of genetic similarity than standardised heterozygosity (Coltman et al., 1999). Relatedness takes into account the population-wide allele frequencies at all loci, whereas standardised heterozygosity only accounts for the mean heterozygosity across all studied loci. This makes a difference, because heterozygosity involving a rare allele is a stronger indication of outbreeding than heterozygosity for two common alleles.

To estimate individual inbreeding based on relatedness, the choice between internal relatedness and relatedness among parents will depend on the data available and on the aim of the experiment. In general, internal relatedness is preferable whenever possible, because it directly estimates average similarity of the maternal and paternal genes present in an individual. In social insects, however, one may want to analyse the effect of colony inbreeding on colony fitness, that is, the fitness consequences of the production of inbred workers when the queen and the male(s) she has mated with are related. In this case, it is usually easier to genotype the queen and her spermatheca than several workers. Using relatedness between parents to study colony inbreeding may also help to avoid a bias. Indeed, if inbred workers have a higher mortality than outbred workers, estimating colony inbreeding based on worker genotypes may lead to an underestimate of colony inbreeding because inbred workers may have died before sampling.

It should, however, be pointed out that estimating inbreeding coefficients for individuals with only a few molecular markers is imprecise (Balloux et al., 2004; Slate et al., 2004). Hence, conclusions based on inbreeding coefficients attributed to single individuals should be treated with caution, unless each individual has been analysed at a large number of loci. However, the estimate is unbiased (Wang, 2002) if relatedness is estimated using the method developed by Queller and Goodnight (1989), and thus it will give a reasonable estimation of the relative ranking of individuals according to their level of inbreeding. Therefore, relatedness-based estimators of the individual inbreeding level are appropriate when studying associations between individual inbreeding and fitness traits. However, whenever possible, a pedigree should be used to estimate individual inbreeding.

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