

Inbreeding avoidance through mate choice

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Introduction

Inbreeding is an often studied phenomenon in biology and its consequences have puzzled researchers for over a hundred years (Darwin, 1876; Haldane, 1932; Sheppard, 1958). This interest stems mainly from its bearing on the applied sciences of plant and animal breeding. During the last couple of centuries, researchers have also started to focus on the impact of inbreeding on natural populations along with its consequences for conservation biology (Waser, 1993; Groombridge *et al.*, 2000). In order to say anything about these applications, it is important to have a foundation of knowledge of the mechanisms which are involved. In this case, the genetics, behaviour and life history that determines the level of inbreeding in natural populations. It is, for example, of great importance to know if the principles and models known from farmed populations can apply to natural systems. One important difference between most managed systems and natural ones is the possibility of individuals to choose their mates in the latter. In order to understand the patterns of inbreeding we must first investigate if and how animals avoid or seek inbreeding by means of mate choice. It is also important to know the mechanisms behind this kind of choice. In this essay I will focus on how mate choice may affect the levels and consequences of inbreeding in natural populations, but before going in to that in detail, it is necessary to introduce the subject of inbreeding in a more general way. I will also start by defining some of the terms that will be used in the rest of the text.

Most of the examples used will be from studies of birds, for two reasons. Firstly, most studies on inbreeding under natural conditions have been performed on an array of bird species and secondly, since I am working with this group of animals myself, it is convenient to use them as examples. I will, in addition, mention studies on other organisms as comparisons to the bird examples.

1. Introducing inbreeding

Inbreeding is a term that is used in many different fields of biological research and other situations. Consequently different authors have used the term in different ways (Shields, 1993). I prefer to use the word inbreeding in its genetic sense, that is, mating between individuals with similar genomes (relatives). This phenomenon is described by Wright's inbreeding coefficient (f), usually interpreted as the probability that two alleles at a specific locus are identical by descent (*ibd*) (table 1) (Charlesworth and Charlesworth, 1987). Since individuals from a small population have a higher probability of sharing alleles by descent, the degree of inbreeding will increase as the population size decreases (Partridge, 1983). To avoid confusion I will use the term incest, instead of inbreeding, for mating within a family. The opposite of inbreeding is called outbreeding. According to the definition above, this would be matings between individuals that do not share many alleles by descent.

Since all individuals in a population, or species, share common ancestors, all matings, according to the definition above, should be inbred (Partridge, 1983). How similar must the genomes of two partners be in order to use the word inbreeding and when is the term outbreeding appropriate? In order to answer this question authors have adopted different thresholds to define inbreeding (or incest). For example matings between first cousins ($r=0.25$) (table 1) or more closely related individuals are sometimes referred to as inbreeding whereas mating between more distantly related individuals is denoted outbreeding (Waser, 1993). The different definitions used have had the consequence that what one author calls inbreeding may very well represent outbreeding to another. Instead of dividing inbreeding into discrete classes it is often more realistic to characterise inbreeding as a continuum (Mitton, 1993) with matings between very close relatives or even self fertilizations at one end of the spectrum and with the extreme outbreeding of inter-species crosses at the other. This is, however, in many instances impractical for statistical and experimental reasons. Whether a continuous variable is used or not is thus a matter of choice and convenience. What one must realise is that inbreeding is a relative concept. Inbreeding must, therefore always be put into a context and it is this context that defines the inbreeding and not the relatedness between mates *per se*. Because of this a reference of some kind must always be used in order to make meaningful statements about inbreeding and outbreeding (Shields, 1993).

1.1 Some genetics behind inbreeding

The standard way of studying inbreeding is to investigate pedigrees from captive populations. Here, the degree of relatedness between mated individuals is generally known, or even manipulated, and coefficients of inbreeding can easily be calculated. When mating occurs between individuals that share recent common ancestors, the heterozygosity of the offspring is decreased and the population genetics deviates from Hardy-Wineberg equilibrium. Inbreeding does not, however, in itself alter the allele frequencies in the population (Lynch, 1991; Hartl and Clark, 1997). As stated above, f describes the level of inbreeding for each individual. Instead F is used to signify the level of inbreeding in a population (table 1). This is defined as the reduction of heterozygosity in an inbred population relative to a random mating population with the same allele frequencies (Wright, 1965).

Table 1. Different coefficients mentioned in the assay.

r:	Coefficient of relationship: The fraction of genes identical by common descent between two individuals.
f:	Wrights inbreeding coefficient: the probability that two alleles at a specific locus are identical by descent.
F:	Population inbreeding coefficient: The reduction of heterozygosity in a population, as a result of inbreeding.
d^2 :	The square of the difference in number of repeats between two microsatellite alleles at a specific locus.

1.2 Observational data

Under natural conditions (the main subject of this essay) observational data is often the only available source of information about kinship between mates. In order to be able to investigate inbreeding in this way, it is necessary to have a very large dataset with a study population that has been followed for several generations. It is uncommon in biology to have such datasets

and consequently these kinds of studies are rare (Shields, 1993). One of the greatest problems with making pedigrees from observational data is the frequent occurrence of extra-pair copulations (epc's) that have been reported from a wide variety of animals (Brooker *et al.*, 1990; Gray, 1997). If a female mates with males other than her observed partner, the paternity of the resulting young will be impossible to assess with purely observational data. One way of getting around this problem is to assess paternity and construct pedigrees by using genetic methods, such as genetic fingerprinting. This approach has been frequently adopted in recent years (Bensch *et al.*, 1994; Kempenaers *et al.*, 1996; Gray, 1997; Peacock and Smith, 1997).

1.3 Genetical data

Development of new laboratory techniques during recent decades has led to the emergence of several new tools for assessing genetic similarity between mating individuals. One of these new measurements is band sharing. (Waldman *et al.*, 1992; Bensch *et al.*, 1994). Many laboratory methods yield results in the form of bands of, for example, proteins or DNA that are separated on a gel or a membrane. The different bands represent different alleles or genes and the number of bands shared between two individuals is a relative measurement of the genetic similarity of these individuals. Similar techniques can be used to assess paternity as discussed in the previous section.

Another measure, mean d^2 (table 1), can be applied when microsatellite data is analysed (Coulson *et al.*, 1998). Microsatellites are segments of DNA with a short sequence motif that is repeated several times at the same locus. The number of repeats and thus the length of a microsatellite evolve very rapidly. The method is based on the assumption that microsatellites evolve in a stepwise manner and compares the number of repeats between two microsatellite alleles. A homozygote at a certain locus has a d^2 of 0. If the alleles of a heterozygote differs by 4 repeat units the d^2 for this locus is 16. The mean d^2 is the average d^2 for all loci in the study. (Coulson *et al.*, 1999). Together with individual heterozygosity the mean d^2 is considered to be a very powerful tool for detecting inbreeding and outbreeding in natural populations (Coltman *et al.*, 1998).

2. Inbreeding depression

2.1 Empirical evidence

The reduced fitness of inbred individuals relative to non-inbred ones is usually called inbreeding depression. The phenomenon is well documented in captive populations and from experiments (Ralls *et al.*, 1979; Lacy *et al.*, 1993; Waldman and McKinnon, 1993), but see Shields (1993) for a critical review. Together with studies of human cognitive performance (Bashi, 1977), this led many authors to believe that inbreeding depression is general and widespread in natural populations. During the eighties and early nineties this standpoint was challenged (Rowley *et al.*, 1985; Craig and Jamieson, 1988; Rowley *et al.*, 1993; Smith, 1993). It was emphasised that the measurements of fitness from captive populations was very different from fitness in nature. Most studies had, for instance, only taken juvenile survival into account and completely ignored other important fitness components. There was thus a demand for more studies on natural populations (Shields, 1993). Some empirical evidence of inbreeding depression in a natural population was found in great tits (*Parus major*) (Greenwood *et al.*, 1978). Gibbs and Grant (1989), however, failed to find any inbreeding depression in Darwins medium ground finches (*Geospiza fortis*) and so did Rowley *et al.* (1985) in superb blue wrens (*Malarus splendens*) (but see Brooker *et al.*, 1990).

In recent years it has been emphasised that inbreeding depression is highly dependent of factors such as the environment (Chen, 1993; Jiménez *et al.*, 1994; O'Brien, 2000), evolutionary history (Bulger and Hamilton III, 1988), age and sex (Coulson *et al.*, 1999) and also that it may vary between years (Keller, 1998). Several studies have now shown inbreeding depression in natural populations, for example in Mexican jays (*Aphelocoma ultramarina*) (Brown and Brown, 1998), great reed warblers (*Acrocephalus arundinaceus*) (Bensch *et al.*, 1994), song sparrows (*Melospiza melodia*) (Keller, 1998; Keller and Arcese, 1998), adders (*Vipera berus*) (Madsen *et al.*, 1999) and harbour seals (*Phoca vitulina*) (Coltman *et al.*, 1998). Studies have also shown that inbreeding depression often is *more* pronounced in natural systems than in laboratory conditions (Chen, 1993; Jiménez *et al.*, 1994). At least two factors have been important for the empirical development during the last century. First, new laboratory techniques, like the ones described above, have made it possible to perform more powerful analysis of inbreeding in nature (Bensch *et al.*, 1994) and second, several long term field studies have been run for enough time to make field data on kinship structures in populations reliable.

2.2 Heterosis

When two inbred lines are crossed with each other the F_1 hybrid often experiences an increase in vigour (Coulson *et al.*, 1999; Edmands, 1999). This phenomenon is usually called heterosis and is generally assumed to be the result of an increased level of heterozygosity due to decreased inbreeding. There is much evidence of heterosis from experiments and breeding on plants in particular (Charlesworth and Charlesworth, 1987) but good evidence of this phenomenon from natural populations seems hard to find. One recent study on red deer (*Cervus elaphus*) has, however, revealed heterosis in interpopulation crosses by using the mean d^2 measurement described in the previous chapter (Coulson *et al.*, 1998). There are also, as will be discussed later, costs involved with too extreme outbreeding.

2.3 Theories of inbreeding depression, genetic load and lethal equivalents

Two theoretical lines of reasoning, overdominance and partial dominance, have been proposed to account for inbreeding depression and heterosis (Charlesworth and Charlesworth, 1987). According to overdominance theory, heterozygotes are generally superior to homozygotes. It has been hard to find any evidence for this model and it is probably not very important as a general force for causing inbreeding depression (Charlesworth and Charlesworth, 1987). Some loci, for example with multiple or fluctuating selection pressures, could however experience overdominance (Mitton, 1993).

The partial dominance theory instead emphasises the expression of recessive deleterious alleles as the cause of inbreeding depression. The existence of these kinds of recessive alleles in a population is often referred to as genetic load (Kirkpatrick and Jarne, 2000). One way of measuring genetic load is by estimating the number of lethal equivalents, this is defined as the number of deleterious genes whose cumulative effect is the equivalent of one lethal gene (May, 1979; Charlesworth and Charlesworth, 1987; Keller, 1998).

There are also possible non-genetical costs of inbreeding and these deal with the production of monomorphic offspring. If young in an inbred clutch are more similar to each other than young in an outbred one, this could lead to more competition between them, because of similar use of limited resources. They would also be equally sensitive towards a changing environment. A clutch with polymorphic young, on the other hand, would experience decreased competition, and it is more probable that at least some of the young would be better

adapted to novel environmental conditions (Bateson, 1983). The different potential costs of inbreeding are summarised in table 2.

2.4 Purging

According to theory, inbreeding would reduce fitness due to increased homozygosity and expression of recessive deleterious alleles. However, after some generations of continuous inbreeding these deleterious alleles would no longer remain in the population and the individuals would experience an increase in fitness (Fowler and Whitlock, 1999). This loss of inbreeding depression due to continuous inbreeding is called purging, and can be seen as a reduction of the genetic load of the population (Barret and Charlesworth, 1991). This could mean that individuals in a regularly outbreeding population would suffer more from an instance of inbreeding than individuals in a regularly inbreeding population (Bengtsson, 1978; Charlesworth and Charlesworth, 1987; Mitton, 1993). One example of this comes from dwarf mongoose in Serengeti, who experience very little inbreeding depression even though incest is very common (Keane *et al.*, 1996). If purging occurs in natural populations this can be taken as evidence for the partial dominance theory, since this model would predict the presence of such a mechanism.

2.5 Outbreeding depression

We have seen that inbreeding can cause fitness reduction in generally outbreeding populations by increasing heterozygosity or by producing monomorphic offspring. There are, however, also potential costs associated with outbreeding (Bateson, 1983) (Table 2). The most striking example of this is the zero fitness experienced by sterile offspring from inter species crosses, but there are also indications of outbreeding depression from matings among populations within the same species (Pusey and Wolf, 1996). Two genetic factors are often cited as the cause of outbreeding depression. The first is loss of epistasis (Waser, 1993; Edmands, 1999), that is the breaking up of coadapted gene complexes through recombination. These effects are often expressed first in the F₂ generation (Lynch, 1991); a phenomenon known in the copepod *Tigriopus californicus* (Edmands, 1999) amongst others. The second genetical cost is the loss of local genetical adaptations. This effect may be most pronounced in sessile organisms (Waser, 1993; Pusey and Wolf, 1996).

There may also be non-genetical costs involved in outbreeding. These are associated with the dispersal to a new area in order to find unrelated partners. Examples of these are the physical

risk and cost of the travel itself, risk of encountering new pathogens (perhaps from the partner) and unfamiliarity of the new area (Bengtsson, 1978). It is also a possibility that skills acquired in the natal area are not appropriate on the new site (Bateson, 1983).

Table 2. Different potential costs involved in inbreeding and outbreeding (Bateson, 1983).

Costs of inbreeding:

Genetical (increased homozygosity):

- Expression of deleterious recessive alleles
- Decreased fitness in overdominant loci

Costs of monomorphic offspring:

- Increased competition between siblings
- No security against a changing environment

Costs of outbreeding:

Genetical:

- Breaking up of co-adapted gene complexes
- Loss of local genetical adaptations

Costs of dispersal:

- Dangerous and exhaustive travel
- Exposure to new sets of pathogens
- Life in new and unfamiliar area
- Acquired skills useful in one area may not be appropriate in another

3. Optimal outbreeding

The negative effects of inbreeding together with those of outbreeding (see previous chapter) led Bateson (1983) to formulate the hypothesis of optimal outbreeding. According to this, animals should try to strike a balance between inbreeding and outbreeding in such a way that the costs of both are minimised (Fig. 1) (Bateson, 1978). This kind of cost vs. benefit approach for studying inbreeding has been adopted by many researchers (e.g. Bengtsson, 1978).

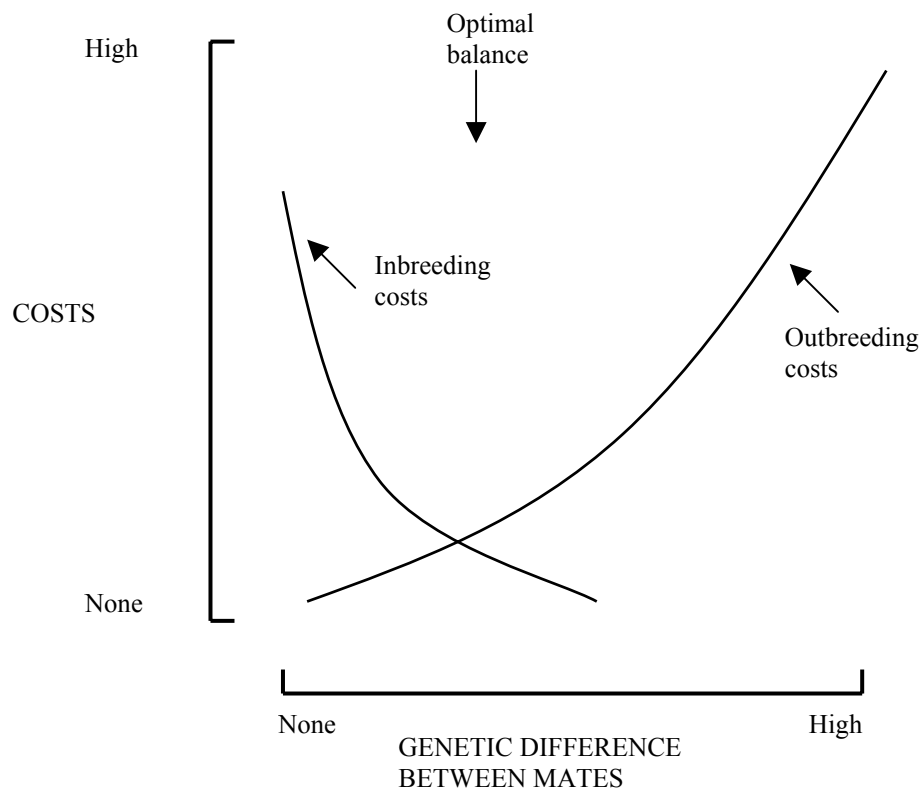


Fig 1. Optimal outbreeding theory. Individuals should balance the different costs of inbreeding and outbreeding in order to minimize the total cost. The optimum is not necessarily at the point of intersection of the lines, but rather where the sum of all costs are lowest. (Figure from Bateson, 1983)

Empirical evidence for optimal outbreeding in nature is scarce because of problems with the interpretation of results. One of the best examples is a study on pikas (*Ochotona princeps*). Here, non-random mating between individuals with intermediate relatedness is recorded (Peacock and Smith, 1997). Several reports (Bateson, 1982; Bolhuis *et al.*, 1988; Krackow

and Matuschak, 1991) have also claimed to provide evidence for the theory by showing that individuals have mating preferences for mates with intermediate relatedness in mate choice experiments (see discussion in the next chapter). According to Bateson (1983), there are two ways in which animals can optimise outbreeding. The first is through natal dispersal - it is common that members of one sex disperse from their home range before they reach sexual maturity (Pusey, 1987). The other mechanism is mate choice through kin recognition, where individuals that look similar, but not identical to close kin are chosen as mates (Fig. 2). The mechanisms of these kinds of behaviours will be examined more carefully in the next chapter.

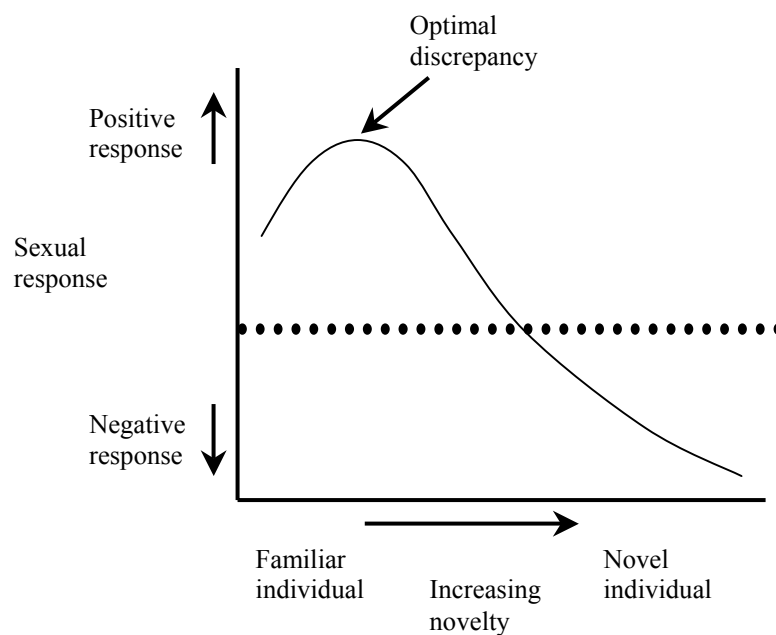


Fig 2. Predicted sexual response to individuals with different familiarity, if mate choice follows the optimal outbreeding theory. The dotted line represents the border between positive and negative response. (Figure from Bateson, 1978)

4. Inbreeding avoidance mechanisms

If inbreeding depression is substantial in a population, mechanisms to avoid inbreeding should be evolutionally favoured (Blouin and Blouin, 1988).

There are different ways for animals to avoid inbreeding. Juveniles can disperse from their natal area before reproduction, reproduction itself can be suppressed in different ways or reproductively active individuals can choose not to mate with relatives (Blouin and Blouin, 1988). I will first give a brief summary of the vast amount of literature of natal dispersal and then take a closer look at different suppression and mate choice mechanisms. It is important to notice that the different mechanisms are not mutually exclusive; rather, several different mechanisms will probably be at work simultaneously and interact with each other.

4.1 Natal dispersal

It is fairly common in nature that individuals of one sex disperses from the home range before reaching sexual maturity. In birds, females are most likely to disperse while in mammals males are usually the dispersing sex (Pusey, 1987). Sex biased dispersal has a strong potential of acting as an inbreeding avoidance mechanism (Greenwood *et al.*, 1978; Pusey, 1987). There is, however, an ongoing debate as to whether inbreeding avoidance is the ultimate cause for the evolution of this behaviour (Moore and Ali, 1984). Several other factors, such as competition for territories or mates, could also explain the phenomenon.

There are many studies on natal dispersal in nature, and I will only give some examples of these. In savannah sparrows (*Passerculus sandwichiensis*) both males and females moved away from the natal territory if the opposite sexed parent was still alive the year after hatching (Weelwright and Mauck, 1998). Inbreeding is reported to decrease in acorn woodpeckers (*Melanerpes formicivorus*) by the dispersal of unisexual sibling groups (Koenig and Pitelka, 1979). Juvenile dispersal as an inbreeding avoidance mechanism is also reported from black-tailed prairie dogs (*Cynomys ludovicianus*) (Hoogland, 1982), white-footed mice (*Peromyscus leucopus*) (Keane, 1990) and olive baboons (*Papio anubis*) (Packer, 1979). This mechanism for inbreeding avoidance might be very easy to evolve since it does not require kin recognition or assessment of genetic similarity. It only involves the *programmed movement* of one sex away from the natal territory. It is, however, a costly mechanism because of the

specific costs involved in the dispersal itself (Blouin and Blouin, 1988). In the following sections I will investigate different ways of avoiding inbreeding without having to bare these costs.

4.2 Reproductive suppression and delayed maturity

One way of dealing with inbreeding depression in animals that live in family groups, is delayed sexual maturation (or other forms of suppression of sexual activity) when the parent of the opposite sex is still present in the group (Pusey and Wolf, 1996). This phenomenon is known in prairie dogs (*Cynomys ludovicianus*), where a young female is significantly less likely to come into oestrus when her father is still living in her natal coterie territory (Hoogland, 1982, 1992). The mechanisms behind this are, as yet, unknown but one possibility is that hormonal activity could be affected by the scent of related individuals (Blouin and Blouin, 1988). In the communally nesting acorn woodpeckers (*Melanerpes formicivorus*) a female does not breed in her natal territory until her father has been replaced. Other females of the same age, however, reproduce after having migrated out from their natal group (Koenig and Pitelka, 1979). No true assessment of genetic similarity is needed for this kind of incest avoidance. Individuals must, however, be able to identify different individuals of their family and some kind of regulation of reproduction in response to this is necessary.

4.3 Direct mate choice

Relative to dispersal, inbreeding avoidance by mate choice is probably a very “cheap” mechanism (Blouin and Blouin, 1988). This is because several of the costs of outbreeding have to do with dispersal itself (Table 2) (Keane, 1990) and these costs can easily be avoided simply by avoiding dispersal. Mechanisms leading to mate choice avoidance of close kin should therefore be favoured by selection under many environmental conditions. Such choice, however, requires some kind of assessment of genetic similarity or relatedness of potential partners. The various ways in which individuals can assess kinship and similarity will be dealt with in the next chapter. Here, I will focus on different kinds of mate choice and their consequences for inbreeding.

In most cases females are the choosier sex, the reason for this being the high reproductive investment of females relative to males. The female simply has more to lose from choosing a bad partner and should thus be careful when choosing a mate (Waser, Austad and Keane, 1986). A typical example of this are lek-systems. Here males gather at arenas (leks) where

they display and defend small territories. Females come to the arena with the sole purpose of choosing a partner and mating with him. After mating the female takes care of all parental duties by her self (Höglund and Alatalo, 1995). Females are, however, not always the most choosy sex. In the case of reversed sex roles, found in some species, the opposite is true. Here, the males have most to gain by avoiding close inbreeding (or extreme outbreeding) and they are thus the ones to choose mates most carefully (Waser *et al.*, 1986). These species provide important possibilities to test different hypotheses about mate choice.

It is important to note that mate choice and dispersal are non-exclusive to one another. Instead, one common driving force of male dispersal is competition for mates. If females prefer to mate with unrelated males, then males should profit from dispersing out of the natal territory to find unrelated females. Such female mediated male migration has been found in for example olive baboons (*Papio anubis*) (Packer, 1979) and white-footed mice (*Peromyscus leucopus*) (Keane, 1990). In this way, female mammals could force males to bear the high cost of inbreeding avoidance (dispersal) by using a cheap mechanism (choice). In birds, females are usually the dispersing sex, a fact that has puzzled investigators of inbreeding avoidance (Pusey, 1987). If the costs of dispersal are smaller in birds than in mammals, this could possibly lead females to disperse themselves instead of forcing the males to disperse by means of mate choice.

When studying mate choice and sexual selection it is often assumed that all females have similar preferences, for example that all females prefer long tails or bright plumage. Recently, researchers have instead started to focus on the importance of individual variation in preferences (Widemo and Saether, 1999). This view is especially important in the case of mate choice for optimal outbreeding and inbreeding avoidance. Here the goal is not to find the most popular of all mates, but rather a partner that is genetically compatible to oneself.

A final important point to make is that the outcome of mate choice is not only dependent of the preferences themselves, but also by how well these preferences can be matched to a potential set of males. If there is only one mate to choose from it is not much use being choosy. In some cases mate choice can be viewed as a “best of a bad job”, where the goal is to find the least bad mate of a set of potential partners. When it comes to pair bonding, other aspects than well matched genes are probably important for mate choice as well.

4.4 Extra pair copulations as an inbreeding avoidance mechanism

In pair bonding species, factors such as territory quality and parental care must be considered along with genetical similarity, when choosing a mate. One possibility for females of pair bonding species is to seek extra pair copulations (epc's) with males having optimal genetical similarities (Pusey and Wolf, 1996). This gives a female the possibility to avoid dispersing but still mate with an unrelated male. One classical example of this kind of behaviour comes from the splendid fairy wrens (*Malurus splendens*). In this species high relatedness within pairs was taken as evidence for close inbreeding (Rowley *et al.*, 1985) until a closer examination by allozyme electrophoresis showed that at least 65% of all young were the result of epc's (Brooker *et al.*, 1990). This mating system resulted in less inbreeding and still secured group living without dispersal costs. A similar strategy seems to be adopted by West African chimpanzees (*Pan troglodytes*) (Gagneux *et al.*, 1999).

The existence of extra pair paternity has been shown in numerous studies and in some cases, for example in red-winged blackbirds (*Agelaius phoeniceus*) (Gray, 1997), females do increase their reproductive success by means of this behaviour. In blue tits (*Parus caeruleus*) the genetic similarity between the female and the extra-pair male was not lower than between the female and her social partner (Kempnaers *et al.*, 1996), so in this case epc is not a method for decreasing inbreeding. One condition which needs to be fulfilled in order to engage in extra-pair copulation is that the female can cheat her social male. If the male knows that he is not the father of any of the young he has nothing to gain from helping with the parental care of the brood.

4.5 Divorce to avoid inbreeding

If the female and her social partner are not genetically compatible with each other and she can not cheat him with extra pair copulations, one way of dealing with the situation would be to get a new partner. Both the female and the male should benefit from a divorce if breeding is unsuccessful. A study of great- and blue tits (*Parus major* and *P. caeruleus*) show that divorce is beneficial if part of the clutch fails to hatch (Kempnaers *et al.*, 1998). The same study, however, fails to find any evidence that the decision of whether to divorce or not was affected by the number of unhatched eggs in the nest.

4.6 Criticism against studies of inbreeding avoidance

When testing inbreeding avoidance on natural populations it is of great importance to have a good null model. It is often not enough to contrast the observed data of mate choice against a model of random mating. Instead, more complex models, taking the distribution of individuals and population structure into account, must be used. Also field experiments may often be necessary in order to draw conclusions about inbreeding avoidance (Pärt, 1996). In addition mate choice experiments have often been accused of not taking important environmental factors into account, and thus may not reveal true mate choice. Instead, the observed pattern may only be artefacts of the experimental set-up (Penn and Potts, 1999).

In addition to this theoretical criticism, there is empirical evidence that inbreeding is often tolerated in nature. There is, for example, no inbreeding avoidance in spite of high inbreeding depression in song sparrows (*Melospiza melodia*) (Keller and Arcese, 1998) and zebra finch (*Poephila guttata*) (Schubert *et al.*, 1989)). In dwarf mongoose (*Helogale parvula*) there is no inbreeding avoidance neither from dispersal nor from mate choice (Keane, 1990). Pärt (1996) concluded that the costs for collared flycatchers (*Ficedula albicollis*) of avoiding inbreeding are greater than those of tolerating it. He also concluded that dispersal to new areas has evolved for other reasons than avoiding inbreeding. Similar conclusions have been drawn by other authors as well (Moore and Ali, 1984; Waser *et al.*, 1986).

5. Kin recognition mechanisms

In order to actively avoid inbreeding by mate choice, animals must have some kind of mechanism for assessing kinship to possible partners. This kind of assessment may be beneficial not only for avoiding inbreeding, but may also be used for knowing when to engage in altruistic behaviour, such as helping. It has also been argued that kin recognition can evolve as a “by-product” of species recognition (Grafen, 1990).

In order to assess kinship, the animal usually uses some phenotypical cue and matches this against some kind of reference. The process of learning this reference is usually known as imprinting, and when it comes to learning the properties that will be used in mate choice we generally speak of sexual imprinting (Ten Cate, 1985). The reference may be the animal itself (self-inspection) or a parent or sibling (familial imprinting) (Pusey and Wolf, 1996).

Alternatively, the animal can learn to recognize the different individuals with whom they grow up and avoid mating with these. In this case, we can no longer speak of true kin recognition since the recognition is independent of the true kinship between the individuals. Instead it is the familiarity of individuals that determines the behaviour (Brown and Eklund, 1994). In many natural situations this mechanism may be sufficient for avoiding inbreeding, for example, by triggering natal dispersal or reproductive suppression. In naked mole-rats (*Heterocephalus glaber*), for example, familiarity is used by females to reduce inbreeding (Clarke and Faulkes, 1999).

5.1 Phenotypical cues

As seen above, phenotypical cues may be used both for true kin recognition (phenotype matching) or for specific recognition of different individuals (familiarity). These cues can be, for example, plumage characters, sounds, odours, pheromones or electrical signals. What kind of signals and cues are used depends highly on what kind of organism it is. Signals that are to be used for true kin recognition must have a high heritability and this limits the number of potential signals. For example, male indigo buntings (*Passerina cyanea*) have dissimilar songs to their fathers and thus in this species, song can not be used as a kinship marker (Payne *et al.*, 1987). In contrast, call characteristics of American toads (*Bufo americanus*) are correlated to genetic similarity and these calls are also used by females to avoid incest (Waldman *et al.*, 1992). Preferences for odours from different related and unrelated individuals have been investigated thoroughly in domestic mice (*Mus musculus domesticus*)

and it seems probable that they use odours to avoid incestuous mating (see for example Egid and Brown, 1989; Krackow and Matuschak, 1991). The genetical background for the signals and recognition mechanisms are often unknown (Waldman *et al.*, 1988). One candidate seems to be the major histocompatibility complex (MHC) genes and these will be discussed in more detail in the next section.

5.2 Major histocompatibility complex

The MHC is a group of genes essential for vertebrate immunity. The function of the MHC is to present antigens to the immune defence system, a presentation that triggers the immune response (Penn and Potts, 1999). There are two main classes of MHC molecules. MHC Class I is involved in the protection against viruses and intra cellular parasites. They present pieces of the pathogen on the surface of the cell, the immune system then destroys the infected cells. Class II MHC instead triggers the antibody response against extra cellular pathogens (Grahm, 2000). Some of the genes in the MHC shows tremendous amounts of polymorphism both within and between populations. High non-synonymous substitution rates of active sites on the MHC genes have been viewed as evidence for balancing selection causing this polymorphism (Hughes and Nei, 1989).

In some species, MHC genes seems to be of importance for mate choice. In mice, for example, (Potts *et al.*, 1991) both males and females prefer mates with a different set of MHC genes than their own. Olfactory cues seems to be important for these preferences, and inbred lab strains of mice are able to discriminate between urine from strains that differ only at MHC loci. Most of the data on mate choice from mice comes from experimental studies in laboratory conditions (Egid and Brown, 1989). There are, however, also reports on MHC dependent mating preferences from semi-natural conditions (Potts *et al.*, 1991). There are also studies on humans indicating similar kinds of preferences (see Penn and Potts, 1999 for a review of these).

In ring-necked pheasants (*Phasianus colchicus*) MHC genotype correlates with male spur length (von Schantz *et al.*, 1996). Male spur length in turn is a secondary sexual character, positively correlated to age, body size and viability. Females also prefer to mate with long spurred males. Female choice is thus under strong influence of MHC genotype in this species (von Schantz *et al.*, 1997).

One possibility is that individuals use the MHC as a basis for kin recognition, and that MHC dependent mate choice is used to avoid inbreeding in general (Grob *et al.*, 1998; Penn and Potts, 1999). It is, however, also possible that this choice aims at the MHC itself. Evolution could favour MHC dependent mating preferences if these give the offspring a better immune defence system. Mating with individuals with a different set of MHC genes than ones own would produce MHC heterozygous offspring. These would be able to cope with a larger set of antigens than homozygous offspring (overdominance hypothesis) (Hughes and Nei, 1992). It would also be preferable to choose mates with specific MHC alleles that are adapted to deal with the pathogens that are present in the specific environment at the time (negative frequency dependent selection) (Brown and Eklund, 1994). Studies have revealed an association between certain MHC alleles and susceptibility to infectious and autoimmune diseases (Briles *et al.*, 1977; Klein, 1986; Hill, 1991).

Conclusions

The effects of inbreeding in natural populations remain puzzling. Inbreeding depression has been demonstrated convincingly in some cases, but in other organisms inbreeding seems to have little or no effect on fitness. Purging is the most commonly used explanation for this, but proof of this mechanism seem hard to find. It seems clear that inbreeding avoidance behaviour is important some times, but in other cases potential costs of such behaviours may exceed the benefits. Finding a balance between outbreeding and inbreeding may be a good strategy but if available partners are scarce this may be hard to do, and individuals must choose a less optimal mate. Nevertheless, I believe that mate choice for optimal outbreeding may be a very important factor for females (and males) when searching for a partner. As techniques for revealing such mate choice improves it will be easier to study in the future. Understanding mate choice is one of the greatest tasks in behavioural ecology and I am convinced that several important answers in this field will be revealed by investigating inbreeding avoidance mechanisms.

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References

- Barret, S. C. H. & Charlesworth, D. 1991. Effects of a change in the level of inbreeding on the genetic load. *Nature* 352: 522-524.
- Bashi, J. 1977. Effects of inbreeding on cognitive performance. *Nature* 266: 440-442.
- Bateson, P. 1978. Sexual imprinting and optimal outbreeding. *Nature* 273: 659-660.
- Bateson, P. 1982. Preferences for cousins in Japanese quail. *Nature* 295: 236-237.
- Bateson, P. 1983. Optimal Outbreeding. In: Bateson, P.(ed), Mate choice. pp. 257-277. Cambridge university press, Cambridge.
- Bengtsson, B. O. 1978. Avoiding inbreeding: at what cost? *J Theor Biol* 73: 439-444.
- Bensch, S., Hasselquist, D. & von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure: nonincestuous inbreeding in the great reed warbler? *Evolution* 48: 317-326.
- Blouin, S. F. & Blouin, M. 1988. Inbreeding avoidance behaviors. *Trends Ecol Evol* 3: 230-233.
- Bolhuis, J. L., Strijkstra, A. M., Moor, E. & van der Lende, K. 1988. Preferences for odours of conspecific non-siblings in the common vole, *Microtus arvalis*. *Animal Behaviour* 36: 1551-1553.
- Briles, W. E., Stone, H. A. & Cole, R. K. 1977. Marek's disease: Effects of B Histocompatibility alloalleles in resistant and susceptible chicken lines. *Science* 195: 193-195.
- Brooker, M. G., Rowley, I., Adams, M. & Baverstock, R. P. 1990. Promiscuity: an inbreeding avoidance mechanism in a socially monogamous species? *Behavioral Ecology and Sociobiology* 26: 191-199.
- Brown, J. L. & Brown, E. R. 1998. Are inbred offspring less fit? Survival in a natural population of Mexican jays. *Behavioral Ecology* 9: 60-63.
- Brown, J. L. & Eklund, A. 1994. Kin recognition and the major histocompatibility complex: an integrative review. *Am Nat* 143: 432-461.
- Bulger, J. & Hamilton III, W. J. 1988. Inbreeding and reproductive success in a natural chacma baboon, *Papio cynocephalus ursinus*, population. *Anim Behav* 36: 574-578.
- Charlesworth, D. & Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237-268.

- Chen, X. 1993. Comparison of inbreeding and outbreeding in hermaphroditic *Arianta arbustorum* (L.) (land snail). *Heredity* 71: 456-461.
- Clarke, F. M. & Faulkes, C. G. 1999. Kin discrimination and female mate choice in the naked mole-rat *Heterocephalus glaber*. *Proc R Soc Lond B Biol Sci* 1995-2002.
- Coltman, D. W., Bowen, W. D. & Wright, J. M. 1998. Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proc R Soc Lond B Biol Sci* 265: 803-809.
- Coulson, T., Albon, S., Slate, J. & Pemberton, J. 1999. Microsatellite loci reveal sex-dependent responses to inbreeding and outbreeding in red deer calves. *Evolution* 53: 1951-1960.
- Coulson, T. N., Pemberton, J. M., Albon, S. D., Beaumont, M., Marshall, T. C., Slate, J., Guinness, F. E. & Clutton-Brock, T. H. 1998. Microsatellites reveal heterosis in red deer. *Proc R Soc Lond B Biol Sci* 265: 489-495.
- Craig, J. L. & Jamieson, I. G. 1988. Incestuous matings in a communal bird: a family affair. *American Naturalist* 131: 58-70.
- Darwin, C. 1876. The effects of crossing and self-fertilization in the vegetable kingdom. John Murray, London.
- Edmands, S. 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. *Evolution* 53: 1757-1768.
- Egid, K. & Brown, J. L. 1989. The major histocompatibility complex and female mating preferences in mice. *Anim Behav* 38: 548-550.
- Fowler, K. & Whitlock, M. C. 1999. The variance in inbreeding depression and the recovery of fitness in bottlenecked populations. *Proc R Soc Lond B Biol Sci* 266: 2061-2066.
- Gagneux, P., Boesch, C. & Woodruff, D. S. 1999. Female reproductive strategies, paternity and community structure in wild West African chimpanzees. *Animal Behaviour* 57: 19-32.
- Gibbs, H. L. & Grant, P. R. 1989. Inbreeding in Darwin's medium ground finches (*Geospiza Fortis*). *Evolution* 43: 1273-1284.
- Grafen, A. 1990. Do animals really recognize kin? *Animal Behaviour* 39: 42-54.
- Grahn, M. 2000. MHC genotype and ornamentation. In: Espmark, Y., Amundsen, T. & Rosenqvist, G.(eds), *Animal signals: signaling and signal design in animal communication*. pp. 421-436. Tapir academic press, Trondheim.

- Gray, E. M. 1997. Do female red-winged blackbirds benefit genetically from seeking extra-pair copulations? *Anim Behav* 53: 605-623.
- Greenwood, P. J., Harvey, P. H. & Perrins, C. M. 1978. Inbreeding and dispersal in the great tit. *Nature* 271: 52-54.
- Grob, B., Knapp, L. A., Martin, R. D. & Azenberger, G. 1998. The major histocompatibility complex and mate choice: inbreeding avoidance and selection of good genes. *Immunogenetics* 15: 119-129.
- Groombridge, J. J., Jones, C. G., Bruford, M. W. & Nichols, R. A. 2000. 'Ghost' alleles of the Mauritius kestrel. *Nature* 403: 616.
- Haldane, J.B.S. 1932. The causes of evolution. Longmans, Green, London, New York.
- Hartl, D.L. & Clark, A.G. 1997. Principles of Population Genetics. Sinauer Associates, Inc, Sunderland.
- Hill, A.V.S. 1991. HLA associations with malaria in africa: some implications for MHC evolution. In: Klein, J. & Klein, D.(eds), Molecular evolution of the major histocompatibility complex. pp. 403-419. Springer-Verlag, Berlin Heidelberg.
- Hoogland, J. L. 1982. Prairie dogs avoid extreme inbreeding. *Science* 215: 1639-1641.
- Hoogland, J. L. 1992. Levels of inbreeding among prairie dogs. *Am Nat* 139: 591-602.
- Hughes, A. & Nei, M. 1992. Models of host-parasite interaction and MHC polymorphism. *Genetics* 132: 863-864.
- Hughes, A. L. & Nei, M. 1989. Nucleotide substitution at major histocompatibility complex class II loci: Evidence for overdominant selection. *Proc Natl Acad Sci U S A* 86: 958-962.
- Höglund, J. & Alatalo, R.V. 1995. Leks. Princeton university press, Princeton.
- Jiménez, J. A., Hughes, K. A., Alaks, G., Graham, L. & Lacy, R. C. 1994. An experimental study of inbreeding depression in a natural habitat. *Science* 266: 271-273.
- Keane, B. 1990. Dispersal and inbreeding avoidance in the white-footed mouse, *Peromyscus leucopus*. *Anim Behav* 40: 143-152.
- Keane, B., Creel, S. R. & Waser, P. M. 1996. No evidence of inbreeding avoidance or inbreeding depression in a social carnivore. *Behavioral Ecology* 7: 480-489.
- Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of Song sparrows (*Melospiza melodia*). *Evolution* 52: 240-250.
- Keller, L. F. & Arcese, P. 1998. No evidence for Inbreeding avoidance in a natural population of Song sparrows (*Melospiza melodia*). *American Naturalist* 152: 380-392.

- Kempnaers, B., Adriaensen, F. & Dhondt, A. A. 1998. Inbreeding and divorce in blue and great tits. *Anim Behav* 56: 737-740.
- Kempnaers, B., Adriaensen, F., van Noordwijk, A. J. & Dhondt, A. A. 1996. Genetic similarity, inbreeding and hatching failure in blue tits: are unhatched egg infertile? *Proc R Soc Lond B Biol Sci* 263: 179-185.
- Kirkpatrick, M. & Jarne, P. 2000. The effects of a bottleneck on inbreeding depression and genetic load. *Am Nat* 155: 154-167.
- Klein, J. 1986. Natural history of the major histocompatibility complex. John Wiley & Sons, Inc.
- Koenig, W. D. & Pitelka, F. A. 1979. Relatedness and inbreeding avoidance: counterplays in the communally nesting acorn woodpecker. *Science* 206: 1103-1105.
- Krackow, S. & Matuschak, B. 1991. Mate choice for non-siblings in wild house mice: evidence from a choice test and a reproductive test. *Ethology* 88: 99-108.
- Lacy, R.C., Petric, A. & Warneke, M. 1993. Inbreeding and outbreeding in captive populations of wild animal species. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 352-374. The university of Chicago press, Chicago.
- Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45: 622-629.
- Madsen, T., Shine, R., Olsson, M. & Wittzell, H. 1999. Restoration of an inbred adder population. *Nature* 402: 34-35.
- May, R. M. 1979. When to be incestuous. *Nature* 279: 192-194.
- Mitton, J.B. 1993. Theory and data pertinent to the relationship between heterozygosity and fitness. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 17-41. The university of Chicago press, Chicago.
- Moore, J. & Ali, R. 1984. Are dispersal and inbreeding avoidance related. *Animal Behaviour* 32: 94-112.
- O'Brien, S. J. 2000. Adaptive cycles: parasites selectively reduce inbreeding in Soay sheep. *Trends in Ecology and Evolution* 15: 7-9.
- Packer, C. 1979. Inter-troop transfer and inbreeding avoidance in *Papio anubis*. *Animal Behaviour* 27: 1-36.
- Partridge, L. 1983. Non-random mating and offspring fitness. In: Bateson, P.(ed), Mate choice. pp. 227-253. Cambridge university press, Cambridge.

- Payne, R. B., Payne, L. L. & Doehlert, S. M. 1987. Song, mate choice and the question of kin recognition in a migratory songbird. *Animal Behaviour* 35: 35-47.
- Peacock, M. M. & Smith, A. T. 1997. Nonrandom mating in pikas *Ochotona princeps*: evidence for inbreeding between individuals of intermediate relatedness. *Molecular Ecology* 6: 801-811.
- Penn, D. J. & Potts, W. K. 1999. The evolution of mating preferences and major histocompatibility complex genes. *Am Nat* 153: 145-164.
- Potts, W.K., Manning, C.J. & Wakeland, E.K. 1991. The evolution of MHC-based mating preferences in *Mus*. In: Klein, J. & Klein, D.(eds), Molecular evolution of the major histocompatibility complex. pp. 421-433. Springer-Verlag, Berlin Heidelberg.
- Pusey, A. & Wolf, M. 1996. Inbreeding avoidance in animals. *Trends Ecol Evol* 11: 201-206.
- Pusey, A. E. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution* 2: 295-299.
- Pärt, T. 1996. Problems with testing inbreeding avoidance: The case of the collared flycatcher. *Evolution* 50: 1625-1630.
- Ralls, K., Brugger, K. & Ballou, J. 1979. Inbreeding and juvenile mortality in small populations of ungulates. *Science* 206: 1101-1103.
- Rowley, I., Russel, E. & Brooker, M. 1993. Inbreeding in birds. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 304-328. The university of Chicago press, Chicago.
- Rowley, I., Russell, E. & Brooker, M. 1985. Inbreeding: Benefits may outweigh costs. *Animal Behaviour* 34: 939-941.
- Schubert, C. A., Ratcliffe, L. M. & Boag, P. T. 1989. A test of inbreeding avoidance in the zebra finch. *Ethology* 82: 265-274.
- Sheppard, P.M. 1958. Natural selection and heredity. Hutchinson, London.
- Shields, W.M. 1993. The natural and unnatural history of inbreeding and outbreeding. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 143-169. The university of Chicago press, Chicago.
- Smith, A.T. 1993. The natural history of inbreeding and outbreeding in small mammals. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 329-351. The university of Chicago press, Chicago.
- Ten Cate, C. 1985. On sex differences in sexual imprinting. *Animal Behaviour* 33: 1310-1317.

- von Schantz, T., Wittzell, H., Göransson, G. & Grahn, M. 1997. Mate choice, male condition-dependent ornamentation and MHC in the pheasant. *Hereditas* 127: 133-140.
- von Schantz, T., Wittzell, H., Göransson, G., Grahn, M. & Persson, K. 1996. MHC genotype and male ornamentation: genetic evidence for the Hamilton-Zuk model. *Proc R Soc Lond B Biol Sci* 263: 265-271.
- Waldman, B., Frumhoff, P. C. & Sherman, P. W. 1988. Problems of kin recognition. *Trends Ecol Evol* 3: 8-13.
- Waldman, B. & McKinnon, J.S. 1993. Inbreeding and outbreeding in fishes, amphibian and reptiles. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 250-282. The university of Chicago press, Chicago.
- Waldman, B., Rice, J. E. & Honeycutt, R. L. 1992. Kin recognition and incest avoidance in toads. *American Zoologist* 32: 18-30.
- Waser, N.M. 1993. Sex, matingsystems, inbreeding and outbreeding. In: Thornhill, N.W.(ed), The natural history of inbreeding and outbreeding. pp. 1-13. the University Chicago Press, Chicago.
- Waser, P. M., Austad, S. N. & Keane, B. 1986. When should animals tolerate inbreeding? *Am Nat* 128: 529-537.
- Weelwright, N. T. & Mauck, R. A. 1998. Philopatry, natal dispersal, and inbreeding in an island population of savannah sparrows. *Ecology* 79: 755-767.
- Widemo, F. & Saether, S. A. 1999. Beauty is in the eye of the beholder: Causes and consequences of variation in mating preferences. *Trends Ecol Evol* 14: 26-31.
- Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395-420.

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