Extra-Pair Copulations and Associated Genetic Benefits

Introduction

Extra-pair copulations (EPCs) occur when females engage in sexual relationships outside of the social pair bond and produce broods of mixed paternity. This promiscuity is prevalent in many species that were previously viewed as monogamous, thus it has become evident that monogamous pairs of animals are not always sexually exclusive. As a result, social monogamy does not reflect sexual or genetic monogamy. In fact, it has been shown that extra-pair offspring are found in approximately 90% of avian species (Griffith et al.).

EPCs with males offer adaptive benefits through direct and genotypic benefits to the female and her offspring. Phenotypic benefits may include parenting assistance and possible courtship feedings by the extra-pair male, whereas genotypic benefits may include acquiring higher quality genes for the offspring, increased genetic diversity of the brood and guaranteed fertilization. Extra-pair mating provides an excellent opportunity to investigate the indirect genetic benefits of female choice, because comparisons between within-pair offspring (WPO) and extra-pair offspring (EPO) can be made. By controlling for non-genetic effects of the rearing environment as well as maternal effects, the differences between the maternal half-siblings should be attributed to the genetic differences between the sires.

For the purpose of this review, I will focus on the controversial literature regarding the genetic benefits of EPCs and how the evolution of female mate choice is influenced. Of particular relevance to EPCs are the Major Histocompatibility Complex (MHC) genes, which have been shown to provide genetic benefits and influence female mate choice in birds (Von Schantz et al.), fish (Lenz et al.) and humans (Chaix et al.).
What are MHC genes and what is their role in EPCs?

MHC genes are found in all vertebrates and are the most polymorphic genes in vertebrate genomes. They play key roles in immune function by coding for cell-surface glycoproteins that control antigen presentation to T-lymphocytes (Griggio et al.). Therefore, measuring levels of polymorphism at these genes can provide indirect measures of the immunological fitness of respective organisms, and immunity level may be an important selection factor for individuals during mate choice. Diversity, or an appropriate number of alleles at the MHC locus increases the potential for an organism to have immunity to more infections, highlighting why the MHC locus is an important criterion in female mate choice.

Extensive research continues to investigate the genetic benefits of EPCs dependent on MHC genes. Most EPCs involve males that have more favorable MHC qualities than individual cuckolded males from the mating pair. This review will delve into the controversial and inconsistent literature surrounding the adaptive significance of EPCs dependent on MHC variability. I will highlight findings that support the idea that there are genetic benefits of MHC-dependent EPCs, as well as the discrepancies regarding the proximate mechanisms for how females select extra-pair males. It is widely agreed that MHC genes are influential factors of female mate choice, yet the mechanisms through which females make their choices remain elusive.

Mechanistic debate: How do females make genetic-based choices for males?

The literature highlighting how females select males with specific genetic characteristics reflects two competing theories. Both theories have been individually supported by studies, but there has also been evidence that they may not be mutually exclusive, and females may use both strategies simultaneously when choosing extra-pair males based on MHC genes. This issue is
relevant for the purpose of this review, as it illustrates the possible mechanisms in which females may select extra-pair males during EPCs.

“Good Genes” Hypothesis

This theory proposes that females gain advantageous alleles (“good genes”) by selecting males with certain phenotypic traits. The first data that directly support a “good genes” hypothesis is presented in a paper that investigates whether male MHC genes correlate with the quality of secondary ornaments in pheasants *Phasianus colchius* (Von Schantz et. al). They sought to determine whether female mate choice based on male spur length reflects how females identify different quality levels of MHC genes in males. They found that MHC heterozygosity confers survival advantages to all male bearers and also increases spur length in males. Females prefer to mate with males that have longer spurs, hence choosing males with increased MHC heterozygosity. This study shows that MHC heterozygosity increases immunity and is associated with the expression of a condition-dependent intersexually selected male trait. Thus, females may increase offspring fitness by selecting extra-pair males based on male secondary ornaments, which are ultimately dependent on MHC characteristics. Overall, females may select extra-pair males using phenotypic indicators of the males’ favorable genes.

Complimentary hypothesis

Although many studies support the idea that EPCs allow females to obtain “good genes” for their offspring, there have also been studies that show no relationship between female mate choice and phenotypic traits that reflect MHC quality in extra-pair males. Thus, the “genetic compatibility” hypothesis offers an alternative explanation for how females perform MHC-dependent mate choice. The premise of this hypothesis is that females make reference to their own respective MHC genotype before selecting a particular male (Griggio et. al). The female
will choose a male that has MHC characteristics complimentary to her own MHC genes in order to either: 1) increase the overall MHC heterozygosity, or 2) obtain the optimal number of MHC alleles for the MHC genotype of the offspring. It is important to highlight the difference in how the female will select a male, based on these two slightly different strategies within the complimentary hypothesis.

Firstly, the if female has low MHC heterozygosity, then the most complimentary set of male MHC alleles will be highly MHC heterozygous. However, it is also evident that females with intermediate MHC heterozygosity have chosen males that also have intermediate MHC heterozygosity, or females with high MHC heterozygosity have selected males with low MHC heterozygosity. This seems counterintuitive at first, especially in relation to the “good genes” hypothesis, or in terms of favoring higher MHC heterozygosity for offspring, however there is a plausible explanation for this behavior. It has been shown in stickleback fish, that MHC complementarity entails females finding appropriate males in order to obtain a favorable number of combined MHC alleles, as opposed to combined MHC diversity (Grigio et. al). Thus a female with intermediate MHC heterozygosity may have a certain number of alleles that will be complimented by a male who also has intermediate MHC diversity, because the number of his MHC alleles combined with hers will form the optimum number of MHC alleles for the offspring. In stickleback fish, there is an optimal number of MHC alleles that mount immune responses, thus the females choose males with MHC genes that are complimentary in number, rather than in diversity, to her own MHC genes (Lenz et. al).

A study with wild Seychelles warblers, Acrocephalus sechellensis, was performed in order to investigate how MHC diversity plays a role in female mate choice in EPCs and how MHC diversity affects lifespan and survival in fledglings (Brouwer et al.). Warblers were
followed for ten years while capture-mark-recapture analyses were used to test for differences between EPO and WPO. It was shown that females prefer to mate with males that had the most dissimilar set of MHC alleles to their own in terms of heterozygosity; females with low MHC diversity selected males that had higher MHC diversity. This shows that females make reference to their own MHC alleles first and choose males that are compatible in terms of MHC heterozygosity. This is known as a “mating up” tactic.

In another study with female house sparrows *Passer domesticus* (Griggio et al), a mate-preference test was conducted in which females with either a low, medium or high number of MHC alleles had to choose between three males with either a low, medium or high number of MHC alleles. Females with a low number of alleles spent the majority of time in front of males with a high number of MHC alleles, while females with a high number of MHC alleles spent the majority of time in front of males with a low number of MHC alleles (Figure 1). Females of intermediate MHC heterozygosity spent the most time in front of males that has an intermediate number of MHC alleles. These observations suggest that females were using an “allele counting” mechanism as stickleback fish do, as opposed to basing their choice on males’ MHC dissimilarity determined by heterozygosity. Although it seems non-adaptive for females with a high number of MHC alleles to choose males with low number of MHC alleles, they may obtain the optimal *number* of MHC alleles for their offspring once their genotypes have combined. Thus, complementarity is not only based on how similar or dissimilar the MHC genes are between the sexes, but also on the number of alleles that each sex possesses at each MHC locus. This explains why we may see differences in female choice of males based on MHC genes. However, both of these strategies within the complimentary hypothesis offer alternative
mechanisms through which females use self-reference before selecting their extra-pair males in EPCs, instead of simply choosing males with “good genes.”

*Both hypotheses play a role in female MHC-dependent mate choice*

Another study has shown that female mate choice in EPCs may be a complex combination of both of the “good genes” and complimentary hypotheses. The relationship between female choice based on MHC dissimilarity (complimentary hypothesis) and the quality of the MHC genotype (“good genes” hypothesis) was tested in female mice (Roberts and Gosling, 2003). Cues of MHC similarity are mediated by urinary odor, thus females may use these cues to distinguish respective MHC similarity in different males during mate choice. However, urinary cues also advertise male status and are thought to be “good genes” indicators of quality; it is costly to mark, thus frequent marking by dominant males is seen ‘honest’ signaling of quality. This study addressed the question of how females weigh both of these cues when making a choice. They measured male urinary marking rates to test for male dominance and then performed female preference tests with these males. Genetic similarity between the respective female and male mice was quantified, thus the extent to which females chose males respectively based on male dominance (“good genes”) and male MHC similarity (complimentary hypothesis) from male urinary scent could be determined. This study is important for female MHC-dependent mate choice in EPCs because it shows the complexity of decision-making when females simultaneously receive both kinds of cue and have to process trade-offs between them. The female’s choice will ultimately be determined by the fitness benefit gained, and this study showed that both good genes and MHC dissimilarity had an effect on female preferences. However, their respective influences can vary depending on the degree of variability in each trait among available males.
These different mechanisms highlighted above offer insight into the complicated and variable ways in which a female may respectively choose an extra-pair male in MHC-dependent EPCs. Based on the studies reviewed so far, it seems unlikely that all females use one standardized method to choose males based on their MHC genotype. Perhaps the method chosen by the female is both context and species-dependent. For future studies investigating MHC-dependent mate choice during EPCs, it is important to consider all mechanisms, non-exclusively, as potential strategies for female choice.

The Benefits of MHC-dependent EPCs

*Optimal MHC number and increased MHC diversity in EPO*

EPCs are costly to females, so the prevalence if this behavior indicates that there must be net benefits of performing EPCs. As this review is focusing on the indirect genotypic benefits, the advantages of EPCs are manifested as increased fitness in offspring through enhanced genetic quality. Comparisons between EPO and WPO have demonstrated that EPCs offer improved fitness through increased MHC diversity or by conferring an optimum number of MHC alleles. In the study previously mentioned with Seychelles warblers (Brouwer et al.), EPO have higher MHC diversity than WPO, because extra-pair males have higher MHC diversity than cuckolded males. Thus, taking part in EPCs is an adaptive strategy performed by the female; it promotes better survival of offspring by ensuring that they obtain higher MHC diversity than the average levels of diversity in the population.

*Increased Immunity in EPO*

It has been widely illustrated that MHC genes play a large role in immunity in vertebrate organisms, especially when individuals express higher MHC heterozygosity or possess an optimal number of MHC alleles. This illustrates why females seek extra-pair sires that will offer
adaptive benefits based on the nature of their MHC genome. The following study gives direct evidence that EPO have higher levels of immunity and ultimately increased survival. In bluethroat birds, *Luscinia svecica*, EPO nestlings have higher immuno-competence (Johnsen et al.). The study involved testing the T-cell reactivity in immune responses of EPO and WPO. Subcutaneous injections of phytohaemagglutinin (PHA) were administered to the offspring; PHA initiates the division of T cells, resulting in various degrees of swelling which indicates the extent of the immune response. The results showed that EPO had higher swelling at the injection sites, thus a higher immuno-competence (Figure 2). This study shows that offspring gain genetic benefits from the extra-pair male, through increased immunity, as a result of MHC-dependent EPCs.

*EPCs Eliminate Negative Inbreeding Effects*

EPCs mostly involve males that are genetically different from the female- especially in terms of MHC diversity (Kempenaers and Dhondt, 1993). Thus, EPCs dependent on MHC heterozygosity reduce the prevalence of inbreeding and the detrimental effects associated with inbreeding depression. Previous studies have shown that inbreeding may cause hatching failure and infertile eggs in blue tits (Kempenaers et al, 1996). EPCs in Savannah sparrows (*Passerculus sandwichensis*) show that females avoid inbreeding by selecting males with more diverse or different MHC alleles. Birds were captured, marked and restriction fragment length polymorphisms (RFLPs) were generated to determine MHC similarity among individuals. The number of MHC-similar pairings in EPCs was determined and offspring were screened to obtain the number of EPO. Results showed that only 8.7% of the females paired with MHC-similar males and it is possible that females avoided MHC similar males to reduce inbreeding (Freeman
et al.). It is important to point out the difficulty in understanding the female’s exact motive when doing studies investigating MHC-dependent EPCs in female mate choice.

A study was also performed with splendid fairy wrens *Malurus splendens* to investigate whether MHC similarity between mates in the social pair influenced the level of EPCs and the extent of inbreeding (Tarvin et al.). Fairy wrens were observed for six years: microsatellite markers were used to determine parents’ genetic similarity and the paternity of the offspring was established. It was shown that the extent of EPCs increased when mates in the social pair were genetically similar (Figure 3). Also, extra-pair males were significantly different from the females at the MHC locus as compared to MHC similarity between the social pair males and females (Figure 4). Additionally, WPO had higher levels of inbreeding and lower MHC heterozygosity than the EPO, thus it is evident that MHC-dependent EPCs reduce the extent of inbreeding.

**Genetic benefits of EPCs may be more complicated to understand: Context-dependent effects**

There have been a plethora of studies done regarding EPCs and genetic benefits in terms of MHC diversity; however, the exact nature of EPCs dependent on MHC is still inconclusive. The studies reviewed so far in this paper offer data in support of the genetic benefits of EPCs, however, not all MHC-based EPCs show the genetic benefits as clearly as these have done. Recent findings have shown that there may be more factors that influence female MHC-dependent mate choice, and the genetic benefits obtained from the extra-pair male may also be obscured by external factors. A comprehensive review of the context-dependency of the genetic effects of EPCs highlights a plethora of studies supporting that external factors influence the genetic benefits of MHC-dependent EPCs (Schmoll, T.).
Environmental variation could obscure the genetic benefits of EPCs

In support of the notion that EPCs provide offspring with genetic benefits, a study using Common yellowthroat birds, *Geothlypis trichas*, illustrated that EPO had superior immune responses compared to WPO (Garvin et al.). However, these results were only significant in the colder of the two years in which the study was performed (Figure 5). In this study, the effects of MHC-dependent mate choice by females in EPCs on offspring immunity were investigated over a two-year period. The numbers of EPO and WPO were obtained, as well as the respective levels of immuno-competence in these two sets of offspring. The results show that in the warmer year, both EPO and WPO had similar immune responses, despite the EPO receiving MHC genes that are supposed to confer higher immunity. This highlights the importance of considering external environmental factors such as weather in studies that investigate the genetic benefits of MHC-dependent EPCs. There may not always be such distinct genetic benefits of EPCs when the environment poses different situations, and understanding this may be important for more accurate identification of the benefits associated with EPCs.

The effects of differential maternal investment with respect to paternity and environmental conditions

There is also mention of the possibility for maternal effects on offspring during care, and these could result from females showing a bias in resource allocation in favor of offspring descending from preferred, genetically superior extra-pair males (Schmoll, T.). This differential maternal investment in WPO and EPO may have the potential to confound paternal genetic effects in offspring fitness in EPCs; higher survival rate of EPO may not only arise from higher quality paternal MHC genes, but also from the mother’s higher investment in the EPO over the WPO.
Environmental variation may also arise from factors such as time of breeding in temperate regions, hatching order and offspring sex. It is thought that these factors may also affect how EPCs occur and the ultimate manifestation of genetic benefits in the offspring. As such, relationships between MHC-dependent EPCs and EPO genetic benefits may not be independent from other external influences. Both maternal and other environmental factors may influence the outcomes of studies highlighting genetic benefits of MHC-dependent EPCs, and this emphasizes the necessity to consider all possible external factors in future studies.

**Difficulties with the study of MHC-dependent EPCs**

*Lack of male phenotypic traits that portray genotypic qualities*

In some cases of MHC-dependent EPCs there are no phenotypic traits or markers of genetic quality that can be directly detected by the female. In contrast to the study involving female mate choice in EPCs and male pheasant ornamentation by Von Schantz et al., a study investigating the relationship between MHC heterozygosity and condition-dependent phenotypic traits found no correlation at all (Promerova, M). This study involved Scarlet rosefinches *Carpodacus erythrinus*; MHC diversity was measured in males and females, and tarsus length, body size and feather ornamentation were quantified in males. The ratio of EPO versus WPO was also obtained and analyses were performed to determine if EPO received genetic benefits as a result of females choosing extra-pair males with MHC heterozygous-dependent traits. There was no relationship between MHC heterozygosity and male tarsus length, body mass or secondary sexual ornamentation in the Scarlet rosefinch. However, there was evidence for higher numbers of EPO sired by fathers with higher MHC heterozygosity. As MHC heterozygosity is not reflected in visible phenotypic characteristics in this species, the females must have other mechanisms for identifying extra-pair males with the higher MHC diversity in EPCs.
Sensing MHC quality through olfaction

There have been many studies illustrating that olfactory cues play a role in advertising MHC characteristics—especially in mammals. Recent research has also shown that birds may possess complex olfactory MHC detection systems as well. There are various mechanisms through which females of different species may select males based on MHC odor cues. Studies highlighting these olfactory mechanisms for MHC-detection offer important information regarding MHC-dependent female mate choice, how this is translated across different species, as well as the ways in which females can identify genetic quality in males.

Urine scents in mice offer cues regarding MHC similarity and male dominance as has been mentioned already (Roberts and Gosling, 2003). In this study, females were able to detect males that were dominant with higher MHC heterozygosity because these males urine-marked more frequently and females could also discriminate between urine odors.

Despite the presence of claims suggesting the absence of a functional sense of olfaction in birds in general, there is now a substantial amount of research demonstrating that a wide variety of avian species are clearly able to detect odors based on electrophysiological and behavioral evidence (Balthazart and Taziaux). This review highlights literature suggesting that birds have olfactory receptor genes homologous to those found in vertebrates, and evidence of brain activation and neural pathways in association with olfactory signals is seen in many bird species as well.

In humans, body odor has been shown to play a role in partner choice, and interesting results have been shown regarding how MHC-dependent mate choice changes when females are on contraceptives (Roberts et al. 2008). In this study, the authors investigated whether contraceptive pill use alters odor preferences using a longitudinal design in which women were
tested before and after initiating pill use. Males and females in the study were genotyped so the MHC similarity could be determined. Females were each matched with pre-selected males: three MHC-similar and three MHC-dissimilar. Males wore white t-shirts to bed for two consecutive nights, which were used to retain the body odor of respective males. During the 10-14 day period of their menstrual cycles, both contraceptive-using and non-contraceptive-using females were asked to smell and rate the t-shirts of their six pre-selected males. Ratings were based on odor pleasantness and desirability of the males. Single women preferred odors of MHC-similar men while women in relationships preferred MHC-dissimilar odors, suggesting that paired females may be seeking to improve offspring quality through extra-pair partnerships. This shows how EPC trends may be similar across species. However, when females were on the contraceptive pill, they preferred the odors of MHC-similar males suggesting that the use of contraceptives alters the mechanisms of female mate choice based on MHC-determined male odors. The contraceptive pill manipulates female hormones, ultimately tricking the body into thinking the woman is pregnant. Thus, females on the pill may not be looking for MHC-dissimilar extra-pair males as seen in most EPCs, but rather men with similar MHC genes. MHC similar males could be more like kin and will likely invest more in caring for the young. This study illustrates that pill use in odor mediated MHC-dependent mate choice may lead to the choice of an otherwise less preferred partner. Without pill use, females usually prefer males with more diverse or an optimal number of MHC alleles. It cannot yet be confirmed, but it is worth noting that perhaps the contraceptive pill influences females to pair with males they would not typically be attracted to. Thus, contraceptive pill use could cause incompatible partnerships, which may be a potential cause of the higher rates of divorce seen in humans today. Regardless, it is evident that
contraceptives influence MHC-dependent mate choice based on olfactory cues in humans, and that females also choose males based on MHC genes.

**Conclusion**

Overall, this review on the genetic benefits of MHC-dependent EPCs illustrates the adaptive function of promiscuity in females of different species. It is a widespread phenomenon—especially among birds, indicating the adaptive nature of the behavior. Although evidence for the genetic benefits has been highlighted here, it is important to note that these benefits are not always as easily found. A surplus of studies has been done regarding the genetic benefits of MHC-based EPCs, but results regarding the adaptive benefits, the mechanisms of female mate choice and environmental effects on both of these are not always consistent. I also agree that a significant reason for the discrepancy in results across many studies is due to the lack of consideration of the effects of extraneous environmental factors (Schmoll, T.). Interestingly, this harkens to the infamous nature-nurture issue, and the fact that the effects of genes and the environment on organisms are not mutually exclusive. For example, there have been numerous studies with strong data sets supporting that EPC females target EP males with higher quality genes, but have inconclusive results regarding the direct genetic differences in the EPO compared to WPO. Additionally, some studies do not offer support that females even choose EP males of higher genetic quality (Schmoll, T.). The results from both of these relatively inconclusive studies regarding MHC-based EPCs may be more supportive if they were re-analyzed in context with the environment. Perhaps there may be interspecies differences in the way EPCs are performed, so these should also be considered when conducting studies.

Furthermore, it would be useful for future studies to consider that the “good genes” hypothesis
and the complimentary hypothesis may not be mutually exclusive in the mechanisms of female mate choice in EPCs.

Future research could possibly investigate aspects of female MHC-dependent mate choice in EPCs that still seem equivocal. Firstly, in the complimentary hypothesis no studies mention how the females actually “count” their own genes and then compliment their own by choosing the male with the appropriate set of MHC genes. What is the specific mechanism used by birds to make self-reference of their own genes? Do they know the characteristics of their own MHC genes by certain phenotypic traits they possess? Or, do birds have a way of knowing the quality of their own immune system, which would be determined by the diversity of their MHC genes? It would also be largely beneficial if we better understood the olfactory mechanisms used by birds to detect MHC characteristics- especially because EPCs are so predominant in this taxon. In humans, interesting results related to how body odor mediates MHC-dependent mate choice have been shown. These results provide a potential direction for future research that could investigate relationships in the community; perhaps studies such as these could provide better information regarding how people can pair with more compatible partners, thus potentially reducing the extent of promiscuity among married couples.

It is incontrovertible that MHC-based EPCs offer genetic benefits to organisms that perform this behavior. This is yet another example of the incredible capacity for animals to overcome adaptive constraints by performing behaviors that on the surface, appear as evolutionary conundrums. EPCs initially seem too costly to be justifiable- especially when the subject of female mate choice in EPCs is on the genetic level. Females must seek extra-pair males with a particular MHC quality, and may end up having more offspring to care for from two different sires. At first this seems unnecessary and energetically expensive, especially
because the female already has a pair bonded mate, but it has been illustrated that the opposite is true. Females are better able to secure the future of their genes and produce genetically superior offspring through MHC-dependent EPCs.


Griggio, M., Biard, C., Penn, D. J. & Hoi, H. 2011. Female house sparrows "count on" male genes: Experimental evidence for MHC-dependent mate preference in birds. BMC Evolutionary Biology, 11.


Figures

Figure 1. Percentage of time spent by focal females in the choice area (mean time ± 1 SE) of stimulus individuals (three males and a control female), according to their individual number of MHC alleles or MHC diversity.

Figure 2. Relationships between residual phytohaemagglutinin (PHA) response of extra-pair young (EPY) and within-pair young (WPY) in bluethroat broods, after controlling for nestling body mass. Maternal half-sibs raised in the same nest (n = 32).
Figure 3. Relationship between genetic similarity (Mean +/- SE) of females to their social mates and level of extra-pair paternity.

Figure 4. Mean +/- SE genetic similarity between females and their social versus extra-pair mates when broods contained only extra-pair young (EPY).
Figure 5. T-cell-mediated immune response (mm) of extra-pair (EPY) and within-pair (WPY) young in 2003 and 2004. Bars indicate least squares means and SE.