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Mate Choice in Scarlet Rosefinch *Carpodacus erythrinus*:

Test of 'Good Genes' and 'Complementary Genes' Hypotheses

Master's Thesis

Praha 2007

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I ABSTRACT

Within the contemporary theory on the sexual selection there are two main competing hypotheses concerning the female indirect benefits arising from the mate choice. The 'Good genes' hypothesis assumes that the female tends to gain for her progeny some generally beneficial alleles through mating with an appropriate male. On the other hand the 'Complementary genes' hypothesis supposes that the mate choice is governed by the female effort to obtain for her offspring the most fitting parental haplotypes. This thesis aimed to provide evidence for discrimination between these two hypotheses in scarlet rosefinch. Three principal aspects of the sexual selection were concerned: (1) the reliability of male ornamentation as an indicator of individual's quality; (2) the associations between parental traits and offspring health indicators and (3) the effect of paternity on offspring heterozygosity and immune responsiveness in mixed-paternity broods. The results indicate that male ornamentation is a reliable signal of individual's health which may be used by females in their mate choice. The ornamentation does not reflect male's heterozygosity. Moreover, the social father's ornamental plumage hue and saturation are good predictors of nestlings' health and immune responsiveness. Nevertheless, there was no effect of extra-pair paternity either on the nestlings' health or swelling response to phytohemagglutinin. Neither the direct comparison of extra-pair young with their maternal half-sibs showed any difference in heterozygosity and immunoresponsiveness. Although these results are more consistent with the 'Good genes' hypothesis than with the 'Complementary genes' hypothesis, the data do not fully support any of them in all details.

Scarlet rosefinch male (photo: M. Vinkler).

ABSTRAKT

Mechanizmy výběru partnera u hýla rudého *Carpodacus erythrinus*: test hypotézy dobrých a komplementárních genů: Teorie pohlavního výběru v současné době poskytuje dvě zásadní hypotézy snažící se vysvětlit nepřímé výhody, které může samice volbou vhodného sexuálního partnera získat. Hypotéza „dobrých genů“ předpokládá, že samice volí svého partnera na základě znaků indikujících přítomnost určitých, v danou dobu vhodných alel, které může samec svým potomkům předat. Hypotéza „komplementárních genů“ naopak tvrdí, že si samice vybírá takového partnera, který nese genotyp co nejkomplementárnější k jejímu vlastnímu. Cílem této práce bylo nashromáždit u modelového druhu hýla rudého data, na jejichž základě by bylo možno rozhodnout mezi platnostmi obou hypotéz. Studovány byly tři hlavní aspekty pohlavního výběru: 1) spolehlivost samčího zbarvení jako indikátoru kvality samce; 2) vztahy mezi znaky rodičů a indikátory zdravotního stavu u jejich potomků; 3) vliv paternity na heterozygotnost a imunitní odpovídavost u mláďat ve hnízdech se smíšenou paternitou. Výsledky této studie ukazují, že samčí ornament představuje u tohoto druhu spolehlivý signál vypovídající o zdraví jeho nositele. Ornamentace není podmíněna celkovou heterozygotností jedince. Dále bylo zjištěno, že různé složky barvy studovaného sekundárního pohlavního znaku samců korelují s některými kondičními a imunologickými znaky u mláďat v jejich hnízdech. Nepodařilo se ale prokázat žádný vliv mimopárové paternity na zdraví či imunitní odpovídavost mláďat, a to ani při přímém srovnání vnitro- a mimopárových mláďat v rámci stejného hnízda. Podobně se vnitropárová mláďata nelišila od svých mimopárových sourozenců ani v heterozygotnosti. Tyto výsledky tedy ve všech svých aspektech nepodporují ani Hypotézu „dobrých genů“ ani Hypotézu „komplementárních genů“.

Scarlet rosefinch nestling 8 days post hatch (photo: M. Vinkler).

II PREAMBLE

The present thesis results from a rich dataset which broadens the research work that has been done by our research group in the last decade (see Albrecht 2004; Albrecht et al. 2007; Poláková et al. 2007a or Schnitzer 2003). Most of the data used in this work originate from field observations and experiments performed during a four-study-season period (2004-2007). All these data were collected by myself, some with the assistance of other members of our team (see acknowledgement). As the amount of work done later in the laboratory much overhangs the possibilities of one person, some of the subsequent analyses of the collected material were processed by other people than me. This fact is always clearly defined within the description of particular methods in the text (with the citation of the thesis within the bounds of which the particular assay was done). The help of these people is also mentioned in the acknowledgement to this thesis. Thus the data resulting from the fruitful cooperation are used here without any illusion of claiming that everything presented in this study was earned by one single person.

I declare that the thesis "Mate Choice in Scarlet Rosefinch *Carpodacus erythrinus*: Test of 'Good Genes' and 'Complementary Genes' Hypotheses" I wrote all by myself on the basis of the material which is cited in the text and with my supervisor's and colleagues' consultations.

Michal Vinkler
Prague, 30th August 2007

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IV General introduction

Ever since the emergence of separate sexes the individual's reproductive success is governed besides the ability of its survival also by the ability of attracting optimal sexual partner. This selection force has presumably promoted the appearance of many phenotypic sex-specific traits, some of which are highly remarkable. Indeed, since the very inception of the modern evolutionary biology the sexual selection is viewed as one of the most powerful evolutionary forces (Darwin 1871). Although much effort has been devoted to the investigation of mechanisms by which the sexual selection operates and valuable theoretical framework has been constituted still our understanding to this topic is only limited as the evidence is rather inconsistent in many details.

This is especially true of our current knowledge concerning the means of mate choice in general and the selection of the partners for extra-pair mating in particular. In contrast to the erstwhile assumptions evidence accumulated showing that in birds and especially in monogamous passerines the occurrence of offspring that is genetically unrelated to the social father of the brood is a widely spread phenomenon (e.g. Møller & Cuervo 2000; Petrie et al. 1998; Westneat et al. 1990). This result of infidelity and extra-pair mating has been recorded in approximately 90% of avian species so far (Griffith et al. 2002). However, the level of extra-pair paternity (EPP) varies significantly among species (see e.g. Kempenaers et al. 1999) creating almost complete continuum: the rates of EPP among broods are referred to range from 0% to 95% (Arnold & Owens 2002). If we take into account only the clearly socially monogamous species (in which EPP is twice as common than in the polygynous species, Hasselquist & Sherman 2001) then the level of EPP occurrence is estimated in average on 11% of offspring and approximately 19% of broods (Griffith et al. 2002). These findings make from the EPP phenomenon an interesting subject for investigation, especially from the point of view of sexual selection strategies and social systems' evolution. EPP represents also an ideal natural model system for the separation of inherited traits from traits determined by the environment as in the same brood and under identical conditions the nestlings differing in the paternal half of their genetical background are reared. This enables us to survey closer the traits used by females to choose sires for their offspring.

Nowadays there does not seem to be concordance in any single universal explanation for the occurrence of the differences in EPP levels. More likely there exists a hierarchical structure of conditions forming the EPP pattern on both interspecific and intraspecific levels (Griffith et al. 2002). Concerning the higher taxonomical level it was found that most of the variation among species can be attributed to the phylogenetic history of the avian lineages (Arnold & Owens 2002) which is often supposed to be linked with conserved differences in life history parameters (Mauck et al. 1999). On a lower level the interspecific and among-population differences in EPP rates are often attributed to demographic factors such as breeding density, breeding synchrony and migration status (Møller & Birkhead 1993; Møller & Ninni 1998; Spottiswoode & Møller 2004a; Strohbach et al. 1998; Stutchbury & Morton 1995; Westneat et al. 1990) or to the genetic diversity within the population (Petrie & Lipsitch 1994; Petrie et al. 1998). Especially the relationship between the EPP level and the species migration status is well-documented. It was predicted that females can gain more from extra-pair fertilizations (EPFs) in migratory species than in sedentary ones as the migration might be associated with the imperfect choice of a within-pair social partner (e.g. due to hasty onset of breeding), greater genetic quality variance in males (due to heterogeneity of environment, parasites or migration strategies) or a better ability of females to assess the male quality (Spottiswoode & Møller 2004a). Indeed, Spottiswoode & Møller (2004a) have shown that the significant part of interspecific variation in EPP rates can be explained by the migration status of the species. Similarly, Albrecht et al. (2007) have recently proved that EPFs tend to be more important for the sexual selection in long-distant migratory passerines.

Before approaching the question of function and evolutionary adaptiveness of EPP we shall consider whose strategy the EPF in fact is. Do males, females or both benefit from engagements in extra-pair copulations (EPCs)? This question is crucial as only if there are any female benefits the EPP can serve as a model system for the female choice in general. The male benefits from EPPs seem to be obvious: EPCs represent a part of male mixed reproductive strategy that enlarges the male's probability of siring additional young ones to those begotten with their own social female-partner (Westneat et al. 1990, see also Kempenaers et al. 1997; Whittingham & Dunn 2005; Albrecht et al. 2007). It is, however, much more difficult to understand correctly possible female benefits. Usually they are supposed to reside in material or more commonly various genetical benefits (see below). It was proposed that especially in socially monogamous species EPFs might be the only way how females could get offspring with chosen males when these are already paired (Westneat et al. 1990; Birkhead & Møller 1993; Hasselquist & Sherman 2001) or when their previous social-partner choice showed to be inappropriate (Jennions & Petrie 2000). However, Arnqvist & Kirkpatrick (2005) have presented impressive evidence supporting the idea that the EPC behaviour does not represent any benefit to females and can be assumed instead as solely male offensive strategy of exclusive fitness enhancement. This evidence is based on the comparison of potential direct costs (especially arising from the lowered social male parental care) and indirect benefits of EPCs for females. The Arnqvist's and Kirkpatrick's (2005) study nonetheless was exposed to severe criticism, especially by Griffith (2007) who claims that their analysis relies on a heterogenic empirical dataset which is, moreover, obviously incomplete and thus unable to cover all potential direct and indirect benefits. Indeed, it is possible that the power of indirect selection on the maintenance of EPP in females estimated on the basis of current knowledge by Arnqvist and Kirkpatrick (2005) may be underestimated (for detailed discussion see Griffith 2007 and Arnqvist & Kirkpatrick 2007; but see also Kempenaers & Sheldon 1996; Petrie & Lipsitch 1994; Westneat & Sherman 1993; Sheldon 2002; Møller 2000; Albrecht et al. 2006). Furthermore, contrary to the claim of Arnqvist and Kirkpatrick (2005) that EPC is for the female the 'best of a bad job', nowadays there is substantial evidence indicating that (at least in some species) females may directly search for EPCs and initiate them (Kempenaers et al. 1992; Birkhead & Møller 1993; Strohbach et al. 1998; Gray 1996; Bouwman et al. 2006; reviewed also in Jennions & Petrie 2000). Dunn and Whittingham (2007) have recently performed a manipulative experiment in tree swallows (*Tachycineta bicolor*) in which they proved that even handicapped females whose flying abilities were lowered by wing clipping search for EPCs. Moreover, it is generally assumed that females may even post-copulatory influence the offspring paternity (Lifjeld & Robertson 1992; Lifjeld et al. 1994; Dunn & Lifjeld 1994; reviewed also by Jennions & Petrie 2000). All this evidence suggests that at least in some cases EPFs may be supposed to bring benefits to females and that the results obtained by Arnqvist and Kirkpatrick (2005) have to be taken with some caution until their further experimental verification. As pointed out by Birkhead and Møller (1993), the mate choice (including the extra-pair mate choice) and resulting paternity have to be regarded as an outcome from a complex interaction of male as well as female strategies that were selected partly by the intersexual arms race and partly by synergistic cooperation between the sexes. This enables us to presuppose that the EPP system represents an adequate model system for the exploration of female pairing strategies. Moreover, as recognized by Griffith et al. (2002) the current hypotheses on the function of EPP are in many aspects identical with the hypotheses aiming to explain the actual evolution of secondary sexual ornaments, which indicates the generality of the female mate choice strategies.

It was proposed that female might seeks for EPPs to obtain non-genetic resources that would directly enhance her fitness (Hypothesis of direct benefits). In its material sense the supportive evidence for this hypothesis is only limited (see Kempenaers et al. 1997; Gray 1997 and also Strohbach et al. 1998 and Blomqvist et al. 2005) and in most species the extra-pair selection according to this hypothesis is unlikely. Jennions and Petrie (2000), however, noted that females receiving material benefits may gain also some genetical benefits at the same time and that is why distinguishing between direct and indirect benefits might be sometimes difficult. Moreover, among the direct benefits one can count also the assurance of egg fertilization for the case or in the case that their own social partner is infertile, which was postulated as the so-called Fertility insurance hypothesis (see Wetton & Parkin 1991 and Sheldon 1994). Some present data nevertheless suggest that infertility may occur only seldom and early embryo mortality is far more often (Birkhead et al. 1995; Kempenaers et al. 1996; Kempenaers et al. 1999; but see Lifjeld et al. 2007).

Hypotheses that follow can be characterised as Hypotheses of indirect benefits to female fitness, which means that the only commodity that the female obtains from EPF is the genetic quality of her offspring. These hypotheses are mostly not mutually exclusive as females may be choosing their partner according to one hypothesis and at the same time profit from the point of view of another one (Kokko 2001; Kokko et al. 2002; Griffith et al. 2002; Charmantier et al. 2004). As early as in 1930s a complex model of evolution of sexual ornamentation through selection on enhanced offspring pairing success was proposed by R. A. Fisher (Fisher 1930). This concept is known as Hypothesis of ‘sexy sons’ and presumes that heritable males’ ornamentation evolves in the response to females’ preferences. Therefore it is convenient for all males to express the preferred ornamentation in the maximal possible level and at the same time females choosing according to any other preference than those shared by the majority of females are penalised through lowered overall fitness due to lower mating success of their male-offspring (see also Pomiankowski 1990). Then females chose their sexual mates in order to provide their male offspring enhanced ornamentation. This ‘Run-away’ model of sexual selection is expected to reach in some point its maximum when the expression of such an ornament is so costly that any improvement is inhibited by natural selection. However, from the solely theoretical point of view this model alone, which may gracefully explain the way of appearance of some ornamentation, lacks the ability to show how the variability in the trait among males does persist. This is crucial because only if there is any variability the preference for the trait as the main cue of the female choice may persist. That is why additional hypotheses are needed to explain the evolution of sexual selection in general and of EPFs in particular. It is therefore probable that the ‘sexy sons’ mechanism takes place aside to some other genetical benefit mechanisms (e.g. the ‘good genes’ mechanism which is described in paragraph IV.1).

Another hypothesis that was proposed to explain the function of EPP is the Genetic diversity hypothesis. It predicts higher average fitness in broods with several genetic fathers than in broods with only one sire due to the increased variability of the offspring from which at least some young may be better adapted to the unpredictable environment (Westneat et al. 1990). Contrary to ‘Complementary genes’ hypothesis (see paragraph IV.2) here it is assumed that females are unable to assess the extent to which they are genetically similar to the males and so there should be no difference in average fitness between extra-pair young (EPY) and within-pair young (WPY) and the distribution of EPP is supposed to be random with respect to broods as well as males. Other presumptions are the existence of high EPP frequencies in all broods in the population and the frequent presence of more than two extra-pair fathers in broods with mixed paternity (see e.g. Griffith et al. 2002 or Charmantier et al. 2004). Though there is some partial evidence in accordance with the Genetic diversity hypothesis (Strohbach et al. 1998; Kempenaers et al. 1999) still the existence of such a model of sexual selection in nature is highly uncertain (see Bouwman et al. 2006). Moreover, as mentioned by Brown (1997) there are some difficulties with recognizing the outcomes resulting from this hypothesis (see also Westneat et al. 1990).

The last two hypotheses that are of special importance for this study are described in detail in separate chapters.

IV.1 'Good genes' hypothesis

Perhaps the most frequently tested hypothesis of indirect female benefits arising from EPP is the Hypothesis of 'good genes'. This is presumably thanks to its intuitively plausible content and relatively approachable testing of its predictions. The 'Good genes' hypothesis assumes that females choose their mates according to some phenotypic traits that serve as honest signals of some desirable additive genotypic trait (Neff & Pitcher 2005). From this point of view, the hypothesis may easily form continuum with the Fisher's 'sexy sons' model as the positive effect of inherited 'good' alleles may either increase the young's survival and viability (as expected by so-called Viability indicator models) or their mating success and fecundity or both and by all these means they can result in superior lifetime reproductive success of the offspring (Kokko et al. 2002; Griffith et al. 2002). Moreover, the indicatory function may be served by several different morphological and colourful ornamental traits which may, however, substantially differ in the information they actually indicate (Gonzalez et al. 1999; Badyaev & Young 2004; Fenoglio et al. 2004). Neglected should be neither the possibility of the post-copulatory cryptic female choice based on molecular traits or sperm viability which may be especially important when there are available no reliable signals for female to obtain information on the ideal mating partner in advance (Jennions & Petrie 2000).

There are several predictions arising from the 'Good genes' hypothesis. First, the hypothesis assumes that the high genetic quality males signalling this by their secondary sexual traits have higher reproductive success than the low quality males. When considering EPFs it means that the high quality males will be more often cuckolders while the low quality males will be commonly among the cuckolded males. Another prediction then is that EPY should be in some aspect better than WPY and that the WPY in the extra-pair male's nest should be no worse then their paternal half-sibs in the mixed-paternity brood. However, the major problem with testing this prediction is that the differential quality of the offspring does not necessarily manifest in the measured characters and so the negative result of a study should not lead to rejection of the hypothesis (Kokko 2001). A substantial body of evidence has shown that at least in some species maternal half-sibs differing in their paternity differ also in quality and that this young quality is indicated by genetical father's secondary sexual traits (Kempenaers et al. 1992; Kempenaers et al. 1997; Sheldon et al. 1997). However, there is also a wealth of contradictious evidence (Krokene et al. 1998; Strohbach et al. 1998; Kraaijeveld et al. 2004; Whittingham & Dunn 2005; Bouwman et al. 2006). These controversial results may suggest that within different species or even different conspecific populations the mate choice leading to EPP may be driven by different purposes.

IV.2 'Complementary genes' hypothesis

The last proposed hypothesis of the female choice evolutionary function in EPCs is the 'Complementary genes' hypothesis known also as 'Compatible genes' hypothesis (see Piálek & Albrecht 2005). It claims that females seek for EPFs to maximize the genetic complementarity between themselves and the sires of their offspring (a non-additive effect on fitness, Neff & Pitcher 2005). By such a choice the offspring would gain the most convenient genome. As a matter of fact, the most heterozygous genome in the offspring is usually understood as the most convenient one. Thus inbreeding avoidance may represent a special type of the 'complementary genes' selection mechanism. As pointed out by Brown (1997) the mate choice based on the prospective offspring heterozygosity and genetic diversity may represent a similar source of genetical benefits to young as the choice based on 'good genes' per se. Indeed, although close inbreeding seems to be very rare in nature (see e.g. Kempenaers et al. (1996), Bensch et al. (1994), Kempenaers et al. (1996) and also Spottiswoode and Møller (2004b) demonstrated significant fitness costs arising from genetic similarity. Moreover, Hansson et al. (2001) have shown in great reed warblers (*Acrocephalus arundinaceus*) and Oh and Badyaev (2006) in house finch (*Carpodacus mexicanus*) a direct relationship between heterozygosity and recruitment success which indicates the individuals survivorship (but for contrary evidence see Edly-Wright et al. (2007).

When concerning EPPs, the 'Complementary genes' hypothesis predicts higher EPP rates in pairs with high genetic relatedness which usually leads to deprived allele richness in young. The overall relatedness between social-pair partners should be then higher than that one between the female and her extra-pair mate. This shall result in higher heterozygosity in the EPY than in the WPY. EPY should be in some aspect better then WPY but they may also be of a higher quality then their paternal half-sibs from their genetical father's nest (depending on the genetical complementarity between the parents of these half-sibs). The hypothesis predicts no consistency among females regarding the choice of their desirable sexual partner (Tregenza & Wedell 2000). However, there is one point that could potentially lead to misunderstanding. The 'complementary genes' based mating definitely does not mean the same as mating with the preference for the most heterozygous male because the second mentioned mating cannot (due to the gene segregation and male and female genome recombination) ensure the highest overall heterozygosity in the offspring.

It is important to answer one question before we accept this hypothesis as a valid one. The complementarity cannot be obviously signalized by optical phenotypic traits which are often assumed to be the most important cues for the mate choice in birds (but see Reid 2007). Are there any other means by which females could recognize the desirable mates? Although the pre-copulatory mate choice is more conspicuous and often discussed, selective mechanisms may also act in copula or post-copulatory (Birkhead & Møller 1993). The best known mechanisms of this kind are the sperm selection and sperm competition (see Birkhead et al. 1993). Some of these mechanisms, if they really occur in birds, might enable the cryptic female choice based on other mechanisms than those proposed by the Indicatory hypothesis. Thus also the sexual selection through 'Complementary genes' might take place in birds. Indeed, Foerster et al. (2006) found that although there was no female preference for unrelated males in within-pair copulations (WPCs) as well as in EPCs still the proportion of young sired by extra-pair males in mixed paternity broods correlated with the genetic relatedness of the social pair mates. Similar mechanisms may be of key importance also in inbreeding avoidance (Tregenza & Wedell 2000).

The supportive evidence for the ‘Complementary genes’ hypothesis consists mainly of the examination of heterozygosity differences between EPY and WPY (Foerster et al. 2003) or the comparisons of parental genetic similarity between social partners who have and those who do not have EPY in their nests (Blomqvist et al. 2002; Fossøy 2007; Lindstedt et al. 2007). In some studies also increased survivorship or other benefits were found in more heterozygous individuals (Foerster et al. 2003; Oh & Badyaev 2006; Lindstedt et al. 2007). Contrary to these results in some studies no effect of genetic relatedness on EPP was found (Kempenaers et al. 1996; Kleven & Lifjeld 2005) and, surprisingly, Ratti et al. (1995) even found in pied flycatcher (*Ficedula hypoleuca*) higher incidence of EPP in genetically more dissimilar breeding pairs.

However, it may be important to note that according to the ‘Complementary genes’ hypothesis there is no need of higher genome-wide heterozygosity of young in the case when the complementarity between mates is required only in some key loci (Brown 1997). The MHC loci are usually considered as one of the best examples of genes that should be concerned by the ‘complementary genes’ selection (Tregenza & Wedell 2000). Females are supposed to choose their mates to maximize the offspring MHC heterozygosity because the heterozygotes are believed to recognize potential pathogens better. Nevertheless, it was proposed that not maximal but optimal heterozygosity in MHC should be preferred (Nowak et al. 1992; Penn & Potts 1999; Milinski et al. 2005). Interestingly, genetic dissimilarity in MHC may reflect the overall genetic dissimilarity and so it can be used as a cue for inbreeding avoidance (Brown & Eklund 1994). Finally, also opposite relationship between genome-wide heterozygosity and heterozygosity in some key loci is possible (Pemberton 2004).

As shown in this brief introduction the contemporary evidence concerning the ‘Good genes’ hypothesis as well as the ‘Complementary genes’ hypothesis is rather equivocal with respect to their validity. In this thesis I therefore aimed to test some of the basic presumptions of these two hypotheses in scarlet rosefinch.

V AIMS AND HYPOTHESES

To bring some new evidence to our understanding of the evolutionary reasons directing the extra-pair mating selection I tried to investigate in this thesis several factors that are likely to determine the mating system in a socially monogamous and highly sexually dichromatic passerine species, scarlet rosefinch, *Carpodacus erythrinus* (for closer description of this species see section VI.2). Special attention was devoted to the possible female genetical benefits arising from the extra-pair mating as the results concerning this topic are currently rather controversial (see e.g. Arnqvist & Kirkpatrick 2005). The very fact of the existence of EPP gives us, by the possibility of comparison of the features of genetical offspring of several males, a unique natural tool for testing the predictions arising from various individual hypotheses concerning a signalling function of animal ornaments.

Generally, this study aims answering (at least partly) three major questions:

1) Does the variation in scarlet rosefinch males' ornamentation indicate any fitness component or genetic quality?
2) Are there any general relationships between the parental traits and brood characteristics?
3) Are there any fitness related differences between the extra-pair young and the within-pair young?

In particular, I hypothesise that:

A. The male ornamentation is an honest indicator of male viability. This presumption is crucial for the validity of Viability indicator hypothesis which is also a part of the 'Good genes' hypothesis.

B. The immune responsiveness is indicated by ornamentation and it is genetically dependent (with respect to heterozygosity). The indication of the immune responsiveness by ornamentation is also in concordance with Viability indicator hypothesis. If it is also determined by individual heterozygosity it is unlikely to indicate 'Good genes' but it rather results from genetic complementarity which is further non-heritable.

C. Parental traits (e.g. parental relatedness or male ornamentation) are predictive to the health and immunological traits of their young. If parental relatedness may predict the health or immune function in young then pairing on the basis of genetical complementarity is likely to occur. On the contrary, if these parameters in young are predicted by male ornamentation it may be supportive for the 'good genes' mechanism rather than genetic complementarity.

D. There are differences in parental and average offspring traits between broods that contain EPY and those that do not. Parental phenotypical traits are supposed to be related to EPY in the case of 'Good genes' hypothesis while social pair relatedness should be higher in EPY broods if 'Complementary genes' hypothesis is correct. Moreover, the enhanced mean brood quality in broods with EPY is expected especially by the Genetic diversity hypothesis.

E. Extra-pair young differ in their immune responsiveness and heterozygosity from their maternal within-pair half-sibs. This result in the case of immune responsiveness is consistent with both 'Good genes' and 'Complementary genes' hypotheses. However, only in the case of 'Complementary genes' hypothesis EPY should be also more heterozygous in average.

VI General methods

VI.1 Study area

This study was carried out on a population of scarlet rosefinch breeding in the Vltava river valley, the National Park Šumava, southern Bohemia, Czech Republic (N 48°48’–48°50’, E 13°55’–13°57’, ~730 m above sea level; for detail information see figure VI.1-1 and VI.1-2) in the period of four study seasons 2004-2007. Each season corresponded to the pre-breeding and breeding period of rosefinches (from the second half of May to early July). The habitat represents three isolated patches of a wet shrubby meadow (the total area of the meadow study sites about 200 ha) surrounded by a rural landscape from one site and hills covered with forest from the other (see figure VI.1-3). All patches of the habitat are very similar in their plant composition. Shrubs represented mainly by spiraea (*Spiraea salicifolia*) and willows (*Salix* spp.) cover 10–20% of total breeding-habitat area. The herbaceous layer consists mainly of meadowsweet (*Filipendula ulmaria*), sedge (*Carex* spp.), and marsh grasses (*Phalaroides arundinacea*, *Glyceria maxima*). The patches are regularly flooded during the April–May period and water persists locally in channels and pools till the beginning of July (Albrecht 2004).

VI.2 Study species – scarlet rosefinch Carpodacus erythrinus erythrinus (Pallas, 1770)

Scarlet rosefinch is a small sized (circa 20g) sexually dichromatic passerine belonging to the subfamily Carduelinae (Cramp et al. 1994). Females and immature males are predominantly olive-brown, reminiscent in their colour to females of house sparrow (*Passer domesticus*) and corn bunting (*Miliaria calandra*), while the adult males are rufescent on the crown, rump and fore-underparts. However, the males are known to vary greatly with respect to their colour in the scale from bright red to drab reddish and even orange (Schnitzer 2003). Moreover, in this species occurs the phenomenon of delayed plumage maturation (Jenni & Winkler 1994), i.e. males acquire the plumage of adults as late as during the moulting in their second year of life while they may be sexually active as soon as in their first year.

The area of distribution of scarlet rosefinch covers mainly the temperate continental climatic zone of the Palaearctic region forming several subspecies of which only *C. e. erythrinus* occurs in Europe (Cramp et al. 1994). This species inhabits predominantly thickets near forest edges, forest clearings, patches of regrowth, and groups of shrubs or isolated trees in humid meadows or river valleys (Cramp et al. 1994). The first rosefinch nest in the Czech Republic was recorded as late as in 1962 and since then the breeding population appears to be growing, counting nowadays approximately 1,300-2,000 pairs of which at least 200 pairs breed in the Šumava region (Šťastný et al. 2006). This trend corresponds with the expansion of the species westwards and northwards in Europe since 1930s (Cramp et al. 1994). The European scarlet rosefinch population is contemporary estimated to 6,100,000-12,000,000 individuals (BirdLife International 2004).

The food of the rosefinches includes mainly seeds, buds and some invertebrates. Contrary to most other passerines even young are fed largely with seeds rather than with insects (Cramp et al. 1994).

This species represents a long-distance migrant with wintering grounds located in the South Asian region, probably particularly in India (Cramp et al. 1994). The extremely long migration causes that scarlet rosefinches spend only a very limited time from May to mid July on their breeding sites in Europe. This results among others in a very specific moulting pattern characterised

◄ Figure VI.1-1: Orientational localization of the study site within the Czech Republic.

▼Figure VI.1-2: Detail localization of the study site.

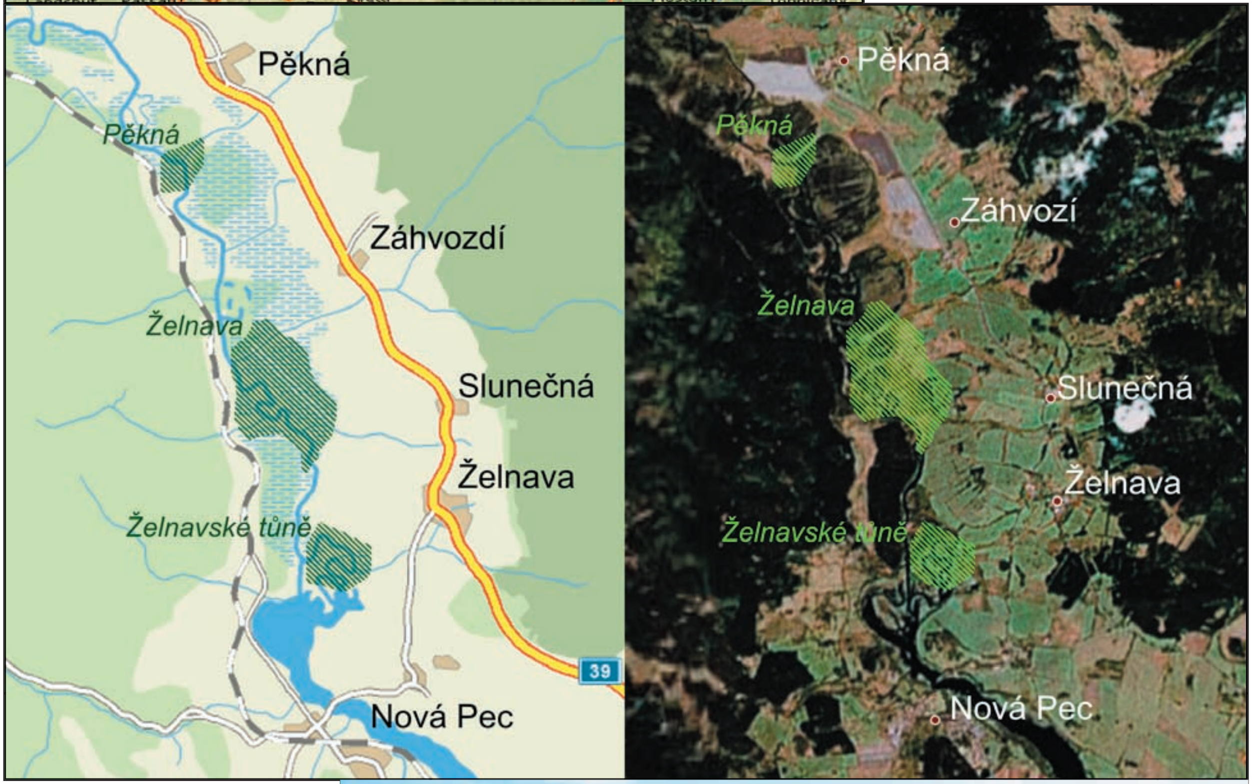


Figure VI.1-3: Želnava study site (photo: M. Vinkler). ►

by moulting after finishing autumn migration in winter, with no moult before the migration (Jenni & Winkler 1994; Cramp et al. 1994). The spring migration is supposed to be rather late (starting in April and early May) and rapid.

In the Czech Republic the breeding season of this species starts in the third decade of May. Males are supposed to be the first to appear on the breeding grounds and in a few days they are followed by females (Björklund 1990). After arrival the males start to attract females by song with display and the pairs are formed as soon as individual females reach the grounds (Cramp et al. 1994). The males do not defend any territory before nesting (Björklund 1990). Scarlet rosefinch represents a socially monogamous species in which the sex-ratio is skewed towards males (Cramp et al. 1994). The presence of EPCs and EPPs in this species has been discovered only recently by our group (Albrecht et al. 2007). Thus, although there does not seem to be any direct female choice in respect to the ornamentation of rosefinch males when pairing (this may be due to an extremely short time for pair making; see Björklund 1990) the reproductive success of individual males may greatly vary. Our data furthermore indicate that the colouration of males correlates with the success in EPFs and the probability of being cuckolded (Schnitzer 2003). Therefore scarlet rosefinch represents a perfect model for testing wide spectra of hypotheses concerning the mate choice and sexual selection.

The rosefinches breed only once per year, with one replacement clutch following failure (Albrecht 2004). The choice of a nest site and nest building is performed by the female (Björklund 1990). In our study population the nests are placed mainly inside the spiraea shrubs. As the species is semicolonial and the males defend only a small area around the nest, mate guarding seems to be the basic mechanism preventing EPC attempts (Björklund 1990). Females lay usually 4-6 eggs in the clutch (Cramp et al. 1994). The eggs are ca. 20.4x14.5 mm large, sub-elliptically shaped and light bluish-green with blackish-brown spotting in their colour. Incubation takes around 11-12 days and the offspring stays in the nest up to 10-13 days of age. Then the fledglings leave the nest being usually still flightless and are fed by their parents for next 2 weeks. While the incubation and heating of the nestlings are provided by females only, young are fed with the contribution of males (Cramp et al. 1994). In our population the nest survivorship ranges between 41% and 83% (Albrecht 2004).

The scarlet rosefinch is a close relative of American house finch (*Carpodacus mexicanus*). It is this species that has been since more that fifteen years used as a model for testing crucial hypotheses concerning the role of carotenoid-based ornamentation in the sexual selection and female preferences in the mate choice. However, although in many aspects similar to scarlet rosefinch, the house finch is a sedentary species with rather low levels of EPP across the broods (Hill 2002; Lindstedt et al. 2007). This genetical proximity of the two species and at the same time very specific differences in several ecological aspects between both of them may enable us direct comparisons that might suggest the nature of mechanisms behind the EPF evolution.

VI.3 Measuring immunity - general remarks to the PHA skin test

Measuring immune responsiveness (widely synonimized as immunocompetence) in wildly living animals represents a great problem of contemporary field immunoecology. In most cases the usage of more sophisticated approaches of classical immunology is precluded by the necessity of avoidance of any harmful or stressful treatments, the minimisation of the time for manipulation with the animal and usually also by the inaccessibility of a well-equipped laboratory near by the field study site. In the past fifteen years the most commonly used approach for measuring cell-mediated immune responsiveness meeting all the limitations became the phytohaemagglutinin skin test (e.g. Lochmiller et al. 1993; simplified by Smits et al. 1999). Although predominantly used in bird studies this method is also applied in veterinary and ecological studies of mammals (Hernandez et al. 2005; de Bellocq et al. 2006; de Bellocq et al. 2007). The test is based on insertion of phytohaemagglutinin (PHA; a potent T cell mitogen, Licastro et al. 1993; Majumdar et al. 1990) subcutaneously into the wing web and induction of measurable swelling response. This reaction was proved to be thymus-dependent (Goto et al. 1978) and the cellular dynamics of the response was described by Goto et al. (1978) and McCorkle et al. (1980) in chicken and recently by Martin et al. (2006) in the house sparrow. It seems that the swelling is caused by inflammatory processes developed by infiltration of macrophages and heterophilic geanulocytes shortly after the insertion of the mitogen which is followed by increased infiltration of eosinophils, lymphocytes and especially basophils within next 24 hours. 48 hours after the treatment the reaction is usually already noticeably decreasing. Contrary to the dynamics of the cell infiltration the total magnitude of the swelling seems to be invariable within the interval between 6 and 24 hours after the administration of PHA into the tissue and there is a positive correlation between several measurements in time (Møller et al. 2003; Navarro et al. 2003). The PHA treatment was proved not to represent any greater source of stress to the studied animals (Merino et al. 1999; Hõrak et al. 2000).

PHA is a plant lectin produced by *Phaseolus vulgaris*. Since the discovery of its polyclonal mitotic effect on T cells by Nowell (1960) PHA is the most widely used mitogen in in vitro studies. PHA is a mixture of 5 isolectins differing slightly in their composition (Leavitt et al. 1977; Felsted et al. 1977). These isolectins are presumably of a tetrameric structure consisting of combinations of two basic subunits with either lymphocyte-specific or erythrocyte-specific binding activities (Yachnin & Svenson 1972; Leavitt et al. 1977; Felsted et al. 1977). Chilson and Kelly-Chilson (1989) provided evidence that PHA binds to T cells with high affinity via their α/β heterodimer or γ chain of the T cell receptor (TCR). This bond triggers the signalisation cascade of TCR irrespectively to its own specificity. Nevertheless, the mechanism by which PHA stimulates the immune response in vivo is not fully understood yet (Kennedy & Nager 2006). The results of Goto et al. (1978) suggest that the full response to PHA is dependent on the well-developed T cell immunity. However, the histological analysis of the reaction provided no evidence that the swelling is caused by proliferation or infiltration of the T cells. It seems that the swelling response is mediated by the infiltration of granulocytes, especially basophils and heterophils (Goto et al. 1978; McCorkle et al. 1980; Martin et al. 2006).

Definitely the PHA response is an expression of some immunological processes (although not well-understood yet) and measurements of its intensity might provide us some valuable basic evidence of the differences in immune responsiveness among individuals or species even when the real nature of mechanisms underlying these differences is not known. Several studies documented the condition-dependent character of the response (Hõrak et al. 2000; Navarro et al. 2003) which offers us the usage of this techniques as a reliable tool for assessing individual quality. Plausibility of this method as a measurement of health and a predictor of individual survival was proved by the results of various recapture (Moreno et al. 2005) and parasitological studies (e.g. Gonzalez et al. 1999; Hawley et al. 2005) although the results of this test must not be considered as a general value of immune function or parasite resistance (Saks et al. 2006; Cheng & Lamont 1988; Ewenson et al. 2001; Blount et al. 2003; Kennedy & Nager 2006; Owen & Clayton 2007).

Nonetheless, there are several methodological aspects that have to be taken into account when using the method for inter-individual comparisons. First, the intensity of the PHA swelling reaction may be modulated by stress prior the treatment (Lazarevic et al. 2000; Ewenson et al.

2003; Morales et al. 2006; but see also Viswanathan et al. 2005) as well as by temperature or food abundance (Lifjeld et al. 2002; Fossøy 2007). That is why it is important to keep all the examined individuals under the same conditions. The response may also vary between sexes (Cheng & Lamont 1988; McGraw & Ardia 2005; but see Lifjeld et al. 2002) and fluctuate in time. It was proved that the intensity of reaction to PHA is age-dependent, being highest in adult individuals and impaired in senescent ones (Lavoie et al. 2007). Bourgeon et al. (2006) report the decrease of intensity of the response during the course of incubation of eggs in common eiders (*Somateria mollissima*) and also Møller et al. (2003) present evidence for variation between breeding and non-breeding individuals. These annual differences in the response may be caused either by the hormonal changes or by the changes in nutritional condition or by the combination of both. Documented is also the temporal variation in the response in the course of a day. When measuring the dynamics of the reaction Navarro et al. (2003) found a greater swelling response occurring during the night. Recently Martinez-Padilla (2006) has reported that the most intensive reaction takes place when the treatment is done in the morning as the magnitude of the response may decrease with the load of physical activity during the day. Unfortunately the generality of this fact across the species is not known yet.

VI.4 Statistical analyses

Generalized linear models were used for most of the analyses. In these models the distribution was approximated to Gaussian (in the analyses of male ptilochronological traits and basophil count VII.3.1, ornamentation VII.3.2, VII.3.3, PHA responses in males VII.3.4, H/L ratio and basophil count in young VIII.3.2 and PHA responses in broods VIII.3.4) or to binomial (in analysis of the H/L ratio in adults VII.3.1, hatching success VIII.3.2 and EPP occurrence IX.3.1). We used generalized linear mixed effect approach to test the influence of various individual nestling traits on the PHA responsiveness (VIII.3.5). In this analysis, identity of nests was treated as random effect. To obtain minimal adequate models, i.e. models with all terms significant (Crawley 2002), candidate models were compared using Mallows' Cp value (add1 and drop functions in S- Plus). In addition, likelihood ratio tests were used to obtain significances of particular terms, with F statistics rather than chi-square statistics used in cases of overdispersion. Non-significant terms were eliminated backward step by step starting with the most complex terms (Crawley 2002). All presented significances are based on Type III Sum of squares. Linear regression was performed in the test of dependence of weight on size (VII.3.1). For the analysis of paired comparisons of EPY and WPY originating from the same broods with respect to their heterozygosity (I.3.2) and PHA responsiveness (IX.3.3) the Paired t-test was performed. In all cases the reliability of the sample distribution approximation to the chosen distribution was tested by One-sample Kolmogorov-Smirnov goodness-of fit test. All statistical analyses of the data were performed in S-PLUS 6.0 Professional and R 2.5.1. software systems. The significance level was set to $p=0.05$ but also slightly non-significant ($p<0.10$) variables were included into the final minimal adequate models.

VII SIGNAL FUNCTION OF THE MALE ORNAMENTATION IN SCARLET ROSEFINCH

VII.1 Introduction

Sexual dichromatism is probably the most conspicuous result of sexual selection. Nevertheless, the question how the ornamental sexual traits appeared is so far difficult to answer. In principle there are four main groups of models describing the evolution of these traits (Jennions & Petrie 2000): (1) Fisher's 'run-away' models dealing with preference-ornament coevolution and advantage of the common female taste; (2) Viability indicator models based on the female ability to judge the viability potential of males (based on heritable 'good alleles' or non-heritable heterozygosity effect); (3) Sensory exploitation models and related; (4) Antagonistic seduction model describing female preferences in the relations to a pre-existing female sensory bias. The evolutionary processes described by all these models are not mutually exclusive and might take place simultaneously in the early evolution of various ornaments. However, at this place at least one more question is equally important: What is the mechanism that preserves the long-lasting within-population variability in the male secondary sexual traits and enables the continuation of the female choice based on the particular trait without fixation of the selected alleles? This question is in fact the core of the so-called lek paradox (see. e.g. Tomkins et al. 2004; Birkhead et al. 2006). The very presence of the intraspecific variation in the well-established secondary sexual traits speaks for the existence of trait expression limitations that are (if not resulting in fixation) in association with genes which are different from those that encode the pure elaboration of the trait. Without these limitations, whatever they are, all males would after an evolutionary short period express their ornaments in the maximal level and females would loose their cue for selection. The limiting factors are supposed to be represented by some general and rather changeable aspects of individual's cooperation with its environment (either social or natural) such as resource acquisition ability (e.g. Hill 2002) or anti-parasite resistance (Hamilton & Zuk 1982). Hence those males who cope better with the cause of limitation shall be able to produce better ornamentation which can be thereafter used by females as an honest signal of the male's genetic quality. This means that the Viability indicator models might offer the explanation for continual selection on secondary sexual traits.

In this respect the emergence of the Parasite mediated sexual selection hypothesis proposed by Hamilton and Zuk (1982) was particularly influential. They suggested that the variation in the ornamental traits is due to the cyclically changing frequency-dependent anti-parasite-resistance advantage of competing genotypes. Thus females choosing a partner according to a given ornament elaboration choose for their offspring in every phase of the cycle different alleles determining the actual anti-parasite resistance. Although this model is rather simplistic (with respect to the complicated architecture of the immune system) it was supported by evidence in many aspects. For example the relationships between ornamentation and parasite load (Thompson et al. 1997; Brawner et al. 2000; Figuerola et al. 2003; Hill et al. 2004; Saks 2004; Fenoglio et al. 2004; Hill & Farmer 2005) and health related traits (Dufva & Allander 1995; Figuerola et al. 1999; Fenoglio et al. 2002a; Fenoglio et al. 2004) were found in various species. Also the genetical heritable nature of the resistance or susceptibility to some diseases is nowadays partly (although fractionally) understood (see e.g. Leveque et al. 2003; Bonneaud et al. 2006) which is also true for the genetical basis of the immunity mechanisms behind this resistance (Lamont & Smyth 1984; Cheng & Lamont 1988; Zhou & Lamont 2003; Buchholz et al. 2004; Bonneaud et al. 2006). The anti-parasite resistance need not be the question of survival, but only of a lowered condition. As shown by many studies mounting an immune response may be costly and even these costs may result in lowered life-time reproductive success (Schmid-Hempel 2003; Faivre et al. 2003a; Alonso-Alvarez et al. 2004; Peters et al. 2004). Therefore genotypes enabling the quicker and easier defeat of the parasite are advantageous.

Many different hypotheses were proposed to explain the direct mechanism linking the ornament elaboration to anti-parasite resistance. Some of them are based on some physiological handicap (Folstad & Karter 1992; Wedekind & Folstad 1994; Hartley & Kennedy 2004; Vinkler and Albrecht in prep.) while others expect some direct trade-off in resource distribution (Hill 2000; Poiani et al. 2000; Peters 2007). However, all these hypotheses assume in fact that the ornament elaboration is usually condition-dependent. The general individual condition is linked to viability which is signalised by the ornamentation. Although the role of 'good genes' is commonly supposed in these processes the condition may be determined by many different genes (e.g. Birkhead et al. 2006). Hence, it is also possible that non-heritable factors such as the individual's genome-wide heterozygosity influence the elaboration of the male secondary sexual traits.

Although the cues for the female choice in EPCs are not fully understood yet, the rate of extra-pair mating was suggested to be positively correlated with the species' level of sexual dichromatism (Møller & Birkhead 1994; Møller 1997). Supportive evidence for the idea that extra-pair mate choice is driven by ornamental selection was presented for instance by Yezerinac and Weatherhead (1997) in yellow warblers (*Dendroica petechia*) or by Krebs et al. (2004) in cattle egret (*Bubulcus ibis*). Interestingly, also Møller and Ninni (1998) found by the metaanalytical approach significant relationship between the elaboration of male ornamentation and the probability of female infidelity. Nonetheless, there is also at least an equally large body of contrary evidence suggesting no effect of ornamentation on extra-pair mating decisions (e.g. Hill et al. 1994 in house finch, Strohbach et al. 1998 in great tit *Parus major*, Cordero et al. 1999 in house sparrow or Kraaijeveld et al. 2004 in black swan *Cygnus atratus*). As it was previously shown by our research group (Schnitzer 2003), in scarlet rosefinch the male colouration may predict the probability of cuckoldry occurrence. These results indicate that the female might engage in EPC to obtain 'good genes'. Therefore this part of our study aimed investigation whether there is any relationship between the male ornamentation on one side and the fitness-related traits on the other side, an essential assumption of the 'Good genes' hypothesis. Special attention was devoted to traits connected with anti-parasite resistance and general health as these associations might suggest the role of parasite-mediated sexual selection. As the viability might be caused by general heterozygosity rather than by particular 'good genes', we investigated the influence of multilocus heterozygosity in 17 microsatellites as well.

We hypothesise that:

A. the male ornamentation is an honest viability indicator:

H.A.1. Male ornamentation is condition-dependent. Supportive evidence for this hypothesis represents the main step to supporting the validity of the Indicatory hypothesis and thus also for the Hypothesis of 'good genes' in sexual selection

H.A.2. This condition-dependence of the ornament elaboration results from differences in individuals genome-wide heterozygosity. The results supporting this hypothesis would be contradictious to the 'Good genes' mechanism as heterozygosity is a non-heritable trait.

B. immunological responsiveness is related to individual ornamentation and genetical traits:

H.B.1. The immune responsiveness is indicated by ornamental plumage traits. In such a case females could assess the anti-parasite resistance potential of the partner on the basis of his ornamentation which is in concordance with 'Good genes' hypothesis of sexual selection.

H.B.2. The immune responsiveness is dependent on individual's heterozygosity. The support of this hypothesis would suggest that the male immunological quality is a non-heritable trait.

VII.2 Methods

The viability indicator hypothesis was tested in adult males captured in 2004-2007 before breeding (i.e. during the period of pairing, approximately between May 16th and 21st) in localities near by Želnava village (Pěkná, Želnava and Želnavské tůně; N 48°50' E13°55', N48°49' E 13°56' and N48°48' E13°57' respectively). All birds were detected according their song and then captured into the mist nets (in some cases using the vocal playback or dummy of a male). Mistneting took place only in the period between 9 a.m. and 3 p.m. to minimize the among-individual differences in the immune response due to circadian variation (see Martinez-Padilla 2006). After the capture each bird was placed into a fabric bag and immediately transported to the field laboratory where it was weighed by means of Pesola gauge (MicroLine 20060, 60g, d=0.5g) and a sample of blood (about 50μl) was collected by puncturing the brachial vein. Thereafter the second outermost rectrix from the left side of the tail was taken for later ptilochronological analysis. In some individuals then the test of immune responsiveness to PHA was performed (see paragraph VII.2.1). The colouration of the individual was documented by digital photograph of the ventral part of the body (for example see figure VII.2.5-1) and the bird was measured (weight and left tarsus length). Tarsus length was used as a general estimate of individual's size (Senar & Pascual 1997). Individual weight was later divided by tarsus length and this weight standardized on size (hereafter referred as mass) was used for further analyses as a condition indicator. All metric measurements were done by digital calliper Kinex , type 6040.2 (accuracy 0,01mm). All measurements were always done by one and the same person only (MV). When all required information was collected the bird was ringed with a standard steel ring of the Czech ringing station (NM Praha) and released. The manipulation time was about twenty minutes.

VII.2.1 Measurements of the immune response

For measuring the immune responsiveness of adult males before breeding only individuals captured in Pěkná and Želnavské tůně (see figure VI.1-2) in 2006 and 2007 were used. As immunity measurement the PHA skin-swelling test according to Smits et al. (1999) was adopted. We performed three independent measurements of the centre of wing web (patagium). This spot was marked with a permanent marker and thereafter hither the injection of 0.10 mg PHA-P (Sigma, product No. L-8754) dissolved in 20.0 μl of phosphate buffered saline (PBS, Sigma, product No. D-5652) was administrated by Hamilton syringe (type 710 RN). Every bird was then placed into an individual cage (41x23x23cm) with access to food and water ad libidum, located in a calm shady place with no visual contact with the surroundings of the cage or with other birds. The birds were periodically controlled to ensure that they feed and do not appear any signs of stress (see Ewenson et al. 2003). After 6 hours (for the usage of 6 hours period see Møller et al. 2003) the magnitude of the wing web in the marked spot was measured, again by three independent measurements. Then collection of other necessary data followed as described above. For the measurements of the thickness of the wing web a digital calliper Kinex, type 6040.2 (accuracy 0.01mm) was used. The close fit of the measuring arm on the skin surface was controlled visually to preserve no deformation of the tissue by the pressure of the calliper. All measurements were always done by one and the same person only (MV) and there was considerably high repeatability among the measurements (r=0.96). For further analyses the mean of these three measurements was used.

VII.2.2 Haematological examination

Blood samples collected from all captured males were used for the preparation of DNA samples and blood smears. The blood smear of each individual was made of one single drop of blood placed on one side of the smear glass and spread along it to the other edge of the smear by a spreading glass. Then the blood was left to dry and the smear was fixed with absolute methanol. Later in the laboratory all smears were stained by Pappenheim's panoptical staining method (3 minutes in concentrated May-Grünwald staining solution, 2 minutes in the same solution diluted 1:1 with distilled water and 15 minutes in Giemsa's solution diluted 1:40 with distilled water, then washed with water and dried). This staining method enables the recognition of all basic blood cell types under the light microscope and so it is convenient for assessment of leucocyte differential counts that may provide useful information about the general health of examined animals (see e.g. Fudge 1989; Hõrak et al. 1998; Ots et al. 1998; El Lethey et al. 2003). All smears were scanned under Opton photomicroscope Axiophot with magnification 1000x to count out the proportion of lymphocytes, heterophils, eosinophils, basophils, monocytes and immature leucocytes within the sample of 100 leucocytes on the smear. In 28 adult rose finches that were not included into the PHA skin-swelling test also the total red blood cells counts, total white blood cells counts were examined and in 42 individuals the haematocrit values were recorded to obtain basic haematological parameters for this species. All counts were performed by MV.

VII.2.3 Examination of the parasite load

The data concerning parasite load were partly obtained from examinations performed by J. Votýpka (for the seasons 2004 and 2005) and partly by MV (2006 and 2007). The method used in this study was described e.g. in Votýpka et al. (2003) and so here I report only its brief description. The blood smears from adult individuals collected in the field and stained according to Pappenheim (see paragraph VII.2.2) were examined with a light microscope at 200x magnification for 5 minutes (equivalent to the observation of 50 microscopic fields). Each smear was thereafter examined for another 10 minutes at 1000x magnification (equivalent to 100 microscopic fields; minimally 10 000 erythrocytes). When no parasites were detected after this time, the smear was considered negative.

VII.2.4 Ptilochronology assays

To measure the differences among individual birds in nutritional condition ptilochronology measurements described by Grubb were used (Grubb 2006; for some modifications in the system of detection of the growth bars see Shawkey et al. 2003). Each individual's second outermost rectrix from the left side of the tail was collected in the field and stored in a paper envelope until the analysis was performed in the laboratory. The rectrices with the 50 mm scale were scanned by scanner Benq (type 5550T) in the gray scale reflex mode with 600dpi resolution. Digital images were adjusted in Corel Photo-paint 12 software by function 'Local Equalization' (with parameters Width 100 and Height 100) which markedly improved the visibility of the growth bars (see figure VII.2.4-1). Thereafter the digital images were used for measurements of the total rectrix length and the mean width of the growth bars in Image Tool version 3 software. For estimation of the mean width of the growth bars a segment of 8 growth bars with the centre in 2/3 of the feather was used. All measurements were done by MV.

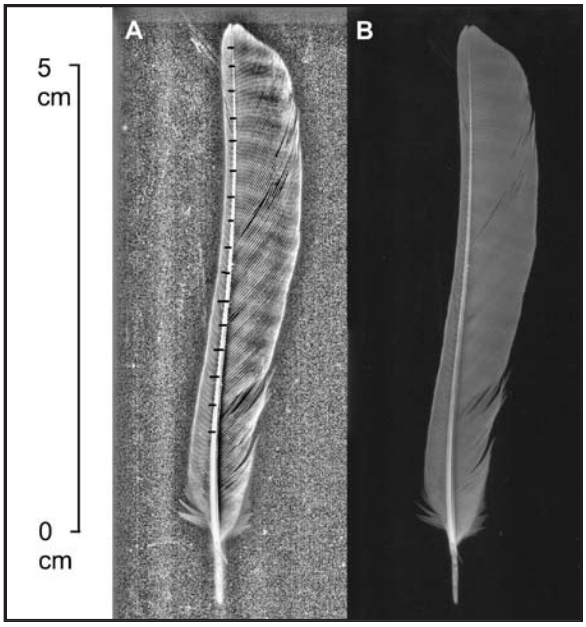


Figure VII.2.4-1:
Transformation of the rectrix image and the visibility of the growth bars (A = the image transformed for measurements, B = the original image).

VII.2.5 Analysis of digital photographs

The data on males' colouration were taken from the present dissertation work of J. Schnitzer (thesis 'Signalization of parent investments in socially monogamous passerines'); they are fully compatible with the dataset in this work and the method of their acquisition is described here only in short. All digital photographs made in the field were done by Olympus digital camera C-765, under standard conditions (light, distance and camera settings) against standard uniform grey background with Kodak colour and grey scale standards (see figure VII.2.5-1). From the photographs breast colour was analysed. Moreover, the photographs were later unified in their general colour in Adobe Photoshop version 10.0 CS.3 according to the colour standards. Thereafter the values of colour were recorded from 12 spots evenly distributed in the central part of the breast ornamentation (elaborated in all individuals). These 12 values represent averages obtained from 5x5 pixel areas of a representative colour. The outputs were noted as average values in colour spaces HSB (hue, saturation and brightness). Hue and saturation were shown to reflect the carotenoid content of the feathers (Saks et al. 2003a) while brightness appears to be determined by the structure of the feather microsurface (Shawkey et al. 2007). All measurements were done by Jan Schnitzer.

Figure VII.2.5-1:
Example of the photograph of ventral part of the rosefinch male body (photo: J. Schnitzer).

VII.2.6 Multilocus heterozygosity assays

Data on genome-wide heterozygosity for this study were kindly provided by Radka Poláková from her work on the dissertation thesis. As the analysed DNA samples were collected in this study the dataset is fully compatible. Originally a multiplex panel for *C. erythrinus* allowing genotyping of 22 polymorphic microsatellite loci was optimized (Poláková et al. 2007a), concretely CE207, CE165, CE 31, CE150, CETC215, CE152, CE67, CE147, CM026, CM014, CM001, CM008E, Hofi 17, Hofi 52, Hofi 23, Hofi 3, Hofi 24, Hofi 7, Hofi 5, LOX 1, LOX 3, LOX 6). However, five loci were excluded (Hofi23, Hofi 7, Hofi3, LOX3 and LOX6) because of the increased frequency of null alleles and hence mean individual heterozygosity was calculated only across 17 loci.

VII.3 Results

VII.3.1 Condition-related traits in adult scarlet rosefinches

To assess the suitable parameters for the further analysis of the factors determining the variance in male colouration we examined first the relationships between various traits that are supposed to be indicative for individual's condition. As tarsus length (hereafter referred as size) was associated with weight (p=0.002) for further analyses we used weight standardized on tarsus length (referred as mass). In order to analyse the correlates of ptilochronology parameters we formulated a generalized linear model in which the rectrix length was dependent on sex, size, mass, *Haemoproteus* occurrence, avian pox occurrence (two-way interactions were included). The resultant minimal adequate model consisted only of sex as predictor of rectrix length (n=88, df=1/86, slope=1.386±SE 0.215, F=41.44 p<0.001). Then this parameter was assumed to be independent within males and was used for further analyses. A similar model for rectrix mean growth bar width was non-significant as a whole (p=0.159) while it was significant for some individual interactions with sex. Therefore we analysed the growth bar width separately for males only. The resulting minimal adequate model was significant only on 90% level (p=0.093) and it is shown in table VII.3.1-1. Again this parameter was considered as independent estimate of individual condition.

Table VII.3.1-1:
Minimal adequate model for growth bar width, n=54, df=3/50, F=2.26 p=0.093.

Variable	Slope ± SE	df	F	p
Growth bar width				
Size	0.288 ± 0.113	1/52	2.67	0.108
Haemoproteus	4.555 ± 2.375	1/51	0.49	0.488
Haemoproteus:size	- 0.218 ± 0.113	1/50	3.74	0.059

The basic description of the differential leucocyte counts examined in 149 adult individuals in the period 2004-2007 is summarized in Table VII.3.1-2. In 2007 also some other haematological parameters were recorded. The haematocrit values ranged between 46% and 65% (median 56%, n=42), the total red blood cells counts varied from 4.13×10^6/µl to 8.47×10^6/µl (median 5.58×10^6/µl, n=28) and total white blood cells counts were approximately estimated to range between $<0.5\times10^3$/µl to 5.58×10^3/µl (median 3.27×10^3/µl, n=10). The mean erythrocyte volume was counted to range between 60fl and 138fl (median 99fl, n=28). The only common blood parasite detected was *Haemoproteus* which was identified where possible as *H. fringillae*.

Table VII.3.1-2:
Differential leucocyte count in scarlet rosefinch (n=149).

Cell type	Range	Mean (%) ± conf. limits	St. deviation	Median
Lymphocytes	4 - 70	28.36 ± 1.98	1.23	26
Immature cells	0 - 6	0.70 ± 0.17	1.04	0
Heterophils	0 - 72	19.10 ± 1.89	11.69	18
Basophils	13 - 86	41.37 ± 2.32	14.33	40
Eosinophils	0 - 28	6.68 ± 0.91	5.64	5
Monocytes	0 - 15	3.80 ± 0.51	3.14	3

Based on the leucocyte differential counts we tried to find out factors associated with the variance in heterophil/lymphocyte (H/L) ratio (a common indicator of health). We set up a generalized linear model in which H/L was proposed to be dependent on sex, mass, size, rectrix growth bar width, rectrix length, period of capture, *Haemoproteus* occurrence and avian pox occurrence (two-way interactions were included). As the most numerous cell type was basophil, we performed a similar analysis also for the basophil count. The minimal adequate models are presented in tables TableVII.3.1-3 and Table VII.3.1-4. However, as there was a significant interaction of *Haemoproteus* occurrence with the period of capture in relation to basophil counts, we decided to test the effect of *Haemoproteus* separately for males captured before breeding as a subset of these males was later used for further analyses. There was a significant positive relationship between *Haemoproteus* occurrence and basophil numbers (t = -2.1356, df = 24, p-value = 0.043).

TableVII.3.1-3:
Minimal adequate model for H/L ratio, n=94, df=4/93, F=2.80, p=0.030.

Variable	Slope ± SE	df	F	p
H/L				
Size	0.178 ± 0.057	1/91	0.39	0.536
Pox	- 6.493 ± 1.183	1/91	1.92	0.169
Period of capture	0.160 ± 0.033	1/91	3.26	0.074
Pox : size	0.314 ± 0.056	1/90	4.41	0.039

Table VII.3.1-4:
Minimal adequate model for Basophil count, n=94, df=6/93, F=8.38, p=0.003.

Variable	Slope ± SE	df	F	p
Basophil count				
Sex	90.886 ± 40.671	1/89	8.88	0.004
Haemoproteus	4.290 ± 1.655	1/89	3.93	0.050
Period of capture	-8.408 ± 1.717	1/89	18.18	<0.001
Rectrix length	-0.942 ± 0.663	1/89	4.12	0.045
Sex : rectrix length	-1.395 ± 0.654	1/87	4.55	0.036
Period of capture : *Haemoproteus*	-4.146 ± 1.665	1/87	6.20	0.015

Figure VII.3.1-1:
Dependence of basophil count on rectrix length and sex (F=female, M=male), n=94. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.

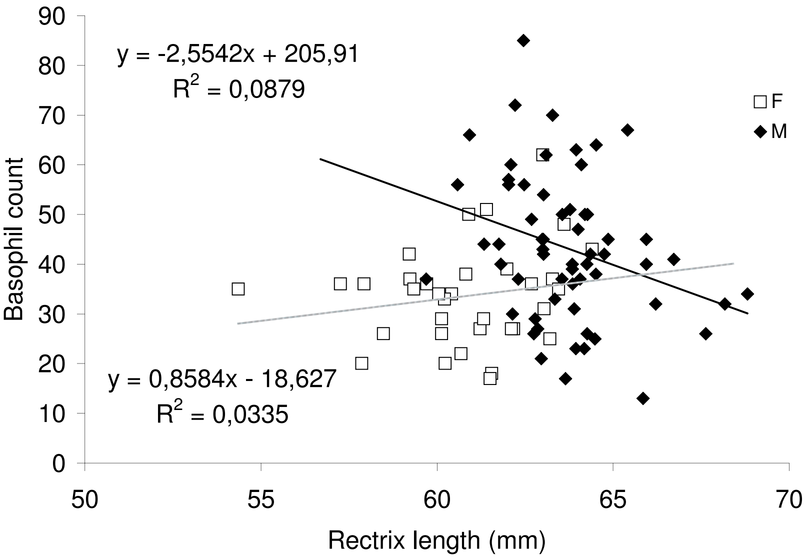
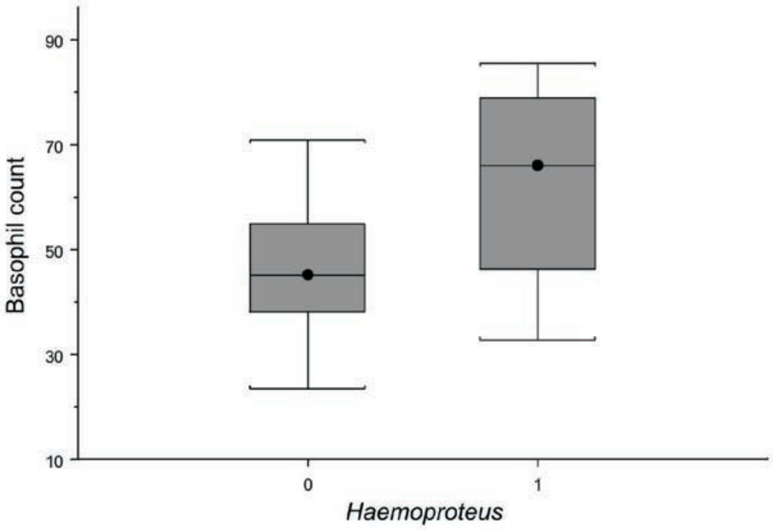


Figure VII.3.1-2:
Association of the basophil count with sex (F=female, M=male), n=94. Boxes indicate quartiles with highlighted median.



VII.3.2 Condition-dependence of male ornamental colouration

After examination of the relationships between condition and health indicators we tested whether the male ornamental coloration is predicted by individual's condition-related traits. On a subset of 26 males captured before breeding in 2005-2007 we tested in three models hue, saturation and brightness separately. These three components of colour were found independent (the absolute value of correlation coefficients in all interactions <0.2, p>0.30). In the generalized linear models we used as independent variables size, mass, rectrix length, mean rectrix growth bar width, Haemoproteus occurrence, avian pox occurrence, H/L ratio and basophil counts. Hue was only non-significantly associated with individual mass (slope=-27.129±SE 14.820, df 1/25, F=3.35, p=0.080). Saturation was not related to any condition parameter (the closest interaction was to H/L ratio with p=0.137). However, brightness was significantly dependent on male's size (p=0.001) and non-significantly on basophil count (p=0.063; minimal adequate model summarised in Table VII.3.2-1).

Table VII.3.2-1:
Minimal adequate model for brightness of the male ornamental trait, n=26, df=2/25, F=8.79, p=0.001.

Variable	Slope ± SE	df	F	p
Brightness				
Size	8.081±2.155	1/24	14.06	0.001
Basophil count	0.152±0.078	1/24	3.83	0.063

Figure VII.3.2-1:
Association of ornamental hue with individual's mass, n=26. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.

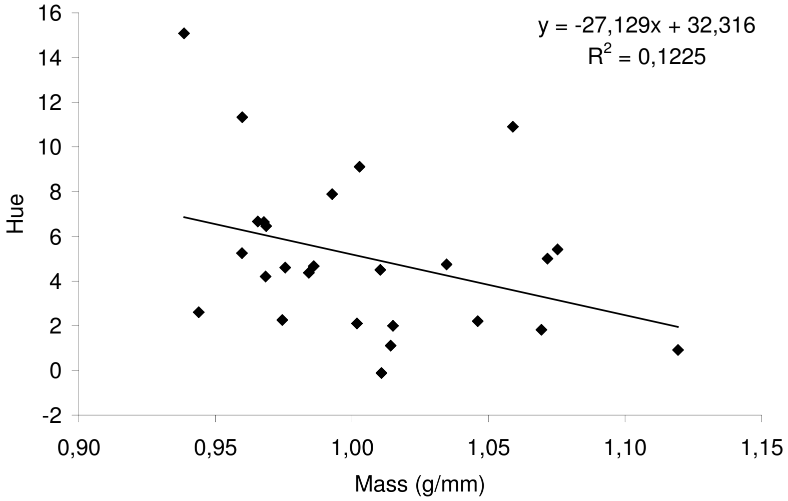


Figure VII.3.2-2:
Association of ornamental brightness and individual's size, n=26. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.

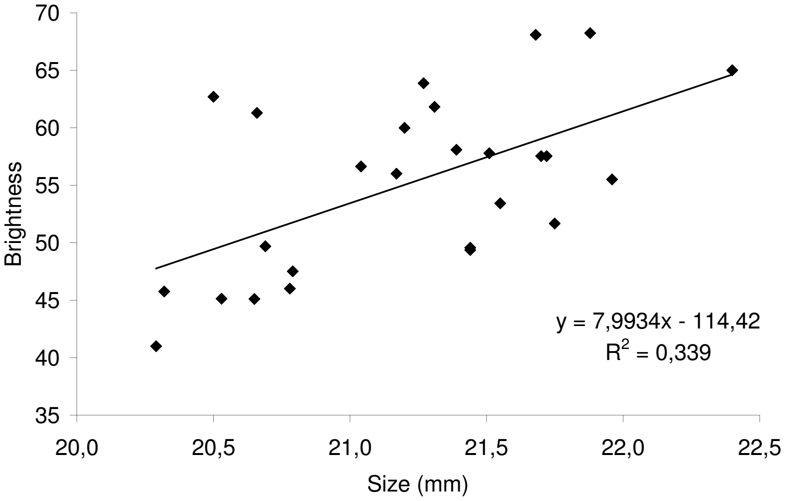
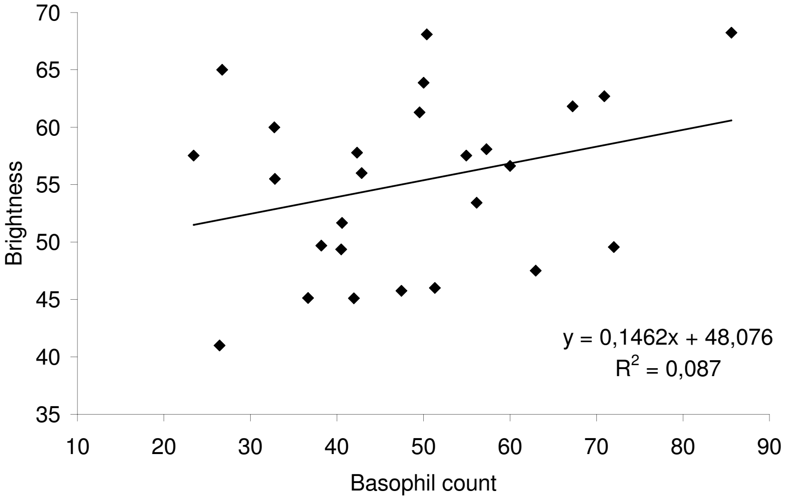


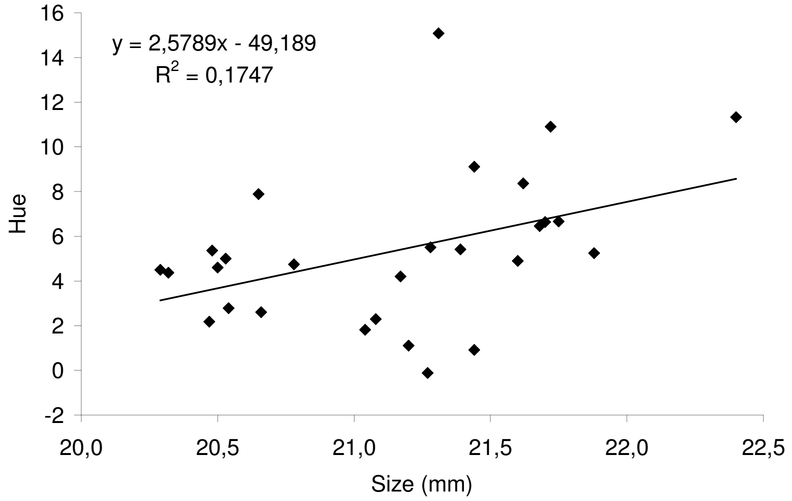
Figure VII.3.2-3:
Association of ornamental brightness and individual's basophil count, n=26. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.



VII.3.3 Heterozygosity and male ornamental colouration

In three models for the individual colour components (hue, saturation and brightness) we tested also the association of the ornament to multilocus heterozygosity. In the model following independent variables were included: heterozygosity, size, mass, *Haemoproteus* occurrence, and two-way interactions. The test was performed on a subset of 28 males captured in the time of mating before breeding (2005-2007) which was however rather different from that used for the previous analysis. Hue was related only to size (slope=2.579±SE 1.099, df 1/27, F=5.50, p=0.027). Saturation was not associated to any independent variable included into this model (the closest interaction was to heterozygosity with p=0.544). Brightness was slightly associated with size (slope=6.110±SE 3.005, df 1/27, F=4.13, p=0.052).

Figure VII.3.3-1:
Relationship between ornamental hue and size, n=28. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.



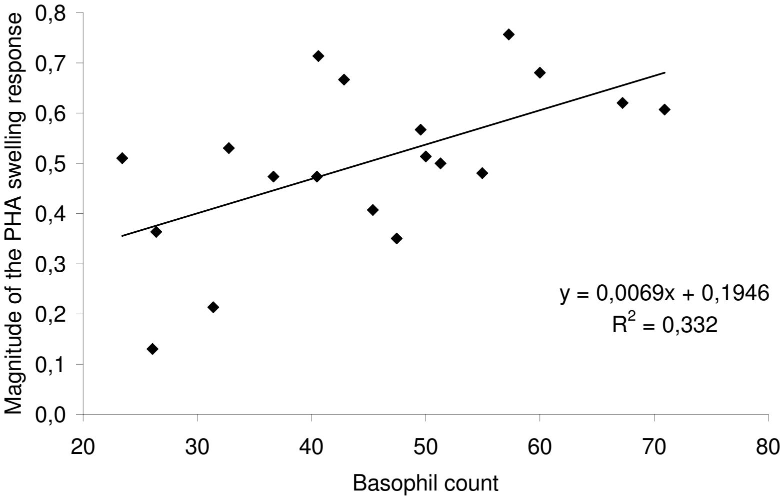
VII.3.4 Immune responsiveness to PHA, heterozygosity and colour of male traits

In a subset of 19 males captured before breeding season in Pěkná and Želnavské tůně localities in 2006 and 2007 we experimentally tested whether the immune responsiveness to PHA is indicated by male colouration (hue, saturation and brightness) and predicted by individual's overall heterozygosity, size and mass. Using the generalized linear model we gained a minimal adequate model which explained as much 43.79% of the variability in immune responses. In this model the PHA responsiveness was associated with male plumage brightness (slope=0.014±SE 0.005, df 1/17, F=8.73, p=0.009) and individual heterozygosity (slope=1.024±SE 0.387, df 1/17, F=6.99, p=0.018). As the brightness was previously shown to be associated with basophil counts we further tested whether the linkage between brightness and PHA responsiveness could be explained on the basis of basophil infiltration into the swollen tissue. After basophil counts were included into the former model a quite different minimal adequate model was gained (see table VII.3.4-1). This model explained 55.33% of the variability among males in their immune responsiveness.

Table VII.3.4-1:
Minimal essential model of PHA immune responsiveness predictors: n=19, df = 3/18 F=6.19 p=0.006.

Variable	Slope ± SE	df	F	p
PHA				
Heterozygosity	0.709±0.357	1/16	3.94	0.066
Size	0.126±0.056	1/16	5.03	0.040
Basophil counts	0.006±0.002	1/16	9.48	0.008

Figure VII.3.4-1:
Association of the basophil count to the magnitude of PHA-induced swelling of the wing web, n=19. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.



VII.4 Discussion

We found that the male plumage colouration is related to several condition indicators. Ornamental hue and brightness are positively associated with male's size. Brightness showed also slightly non-significant tendency for positive correlation with basophil counts and hue tended to be negatively associated with male mass. We detected no association of the plumage colouration with genome-wide heterozygosity. However, heterozygosity together with ornamental brightness predicted the individual magnitude of the swelling immune response to subcutaneous application of PHA from more than 40 %.

Surprisingly, contrary to the observations in closely related house finch (Hill & Montgomerie 1994) we did not find any relationship between plumage colouration and nutritional condition estimated on the basis of ptilochronology parameters. Similarly, our data show only limited relationship of ornamental colour to other commonly used health indicators (such as H/L ratio Dufva & Allander 1995; Figuerola et al. 1999; Saks et al. 2003b; Loyau et al. 2005). In house finch it was shown that ornamental colour signalises the parasite resistance (Thompson et al. 1997; Hill & Farmer 2005). In our study we failed to find any relationship between pox occurrence and the decrease of the males' ornamental colour as reported by Thompson et al. (1997) and there was neither any linkage of colouration to *Haemoproteus* infection. For instance Hõrak et al. (2001) showed that in adult tits birds infected with *Haemoproteus* have increased ornamental hue in comparison with the non-parasitized individuals. The lack of similar association between ornament and disease occurrence in our case could be caused by only low health impact of the *Haemoproteus* and pox infestation on birds in our study population. However, pox occurrence was found to alter the H/L ratio with respect to size. This might be due to elevated lymphocyte levels in afflicted individuals which we have recently described together with MHC class I-allele-number-based resistance to avian pox in this species (Vinkler et al. 2007). The results of this study moreover suggest that size may influence the quantity of response to this disease.

We detected a clear tendency for redder birds to have a larger body mass which is consistent with the hypothesis that colour hue might honestly signalise the food acquiring ability of the ornament bearer (Hill 1992; Hill et al. 2002). We also found that ornamental hue is significantly higher in larger males. This means that larger birds were generally less red than the smaller ones. As size might be associated with dominance and dominance was found in house finch to be higher in drabber birds (McGraw & Hill 2000) it is possible that the hue component of colouration might also reflect some dominance-related traits such as aggressivity or social status. Plumage brightness was in this study positively related to size as well. This might suggest that also brightness reflects male quality. Currently the available evidence for the significance of the brightness component of colour in the plumage signal function is only very limited and even this evidence is rather equivocal (see e.g. Figuerola & Senar 2007). We found a tendency for positive relationship between basophil levels in blood and plumage brightness. Interestingly, in our rosefinch population there were unusually high proportions of basophils among peripheral blood leucocytes. Although the data on the variability in basophil levels in peripheral blood of wild animals are very rare, the results of some studies (e.g. Friedl & Edler 2005) suggest that the proportion of basophils may be in fact in some species much higher than are the normal physiological values known from domestic chicken. The basophil function in birds is so far only poorly understood but on the basis of comparison with mammals it is supposed that this cell type participates in acute inflammatory defence (Daloia et al. 1994; Maxwell & Robertson 1995; Campbell & Ellis 2007). It is also known that basophils are induced to circulate in the blood stream in higher numbers by stress (Maxwell 1993; Altan et al. 2003; Campbell & Ellis 2007; Bedanova et al. 2007). However, for several reasons our results are very unlikely to be caused by stressful manipulation with the birds. In the field all blood samples were collected within half an hour after the capture and prior to any further manipulation with the examined individual. It was earlier described by Davis (2005) in house finch that the handling time shorter than 1 hour does not influence the differential leucocyte counts on smears from peripheral blood. Scope et al. (2002) moreover showed that even 3 hours following after the stress event there are no significant changes in basophil ratio in peripheral blood. Stress is known to increase especially H/L ratio (Lazarevic et al. 2000; Ewenson et al. 2001; Scope et al. 2002; Ruiz et al. 2002; El Lethey et al. 2003; Bedanova et al. 2007) and we did not find any unusually high proportions of heterophils in our smears. As similar proportions of basophils in blood were recorded also in nestlings in which no potentially stressful capturing was performed, it is possible that the high levels of basophils are in this long migrating passerine a part of a particular anti-parasite defence. This is supported also by our finding that higher basophil levels were recorded in birds suffering from *Haemoproteus* infection and in birds in a worse nutritional condition (having shorter tails). Although *Haemoproteus* occurrence alters the health state as well as blood parameters in infected individuals (Garvin et al. 2003) it is unlikely that the basophil levels were elevated directly due to the presence of this blood parasite as in other avian species the response to *Haemoproteus* elevates especially lymphocytes and not so much basophils (Ots & Hõrak 1998). More probably both *Haemoproteus* and basophil counts reflect the general health state that is influenced by some other disease. Hence it seems that higher quality birds may maintain lower basophil counts when resisting the parasite intrusion. Therefore birds with higher brightness are probably in a generally worse condition. This is directly an opposite view on the brightness signal function than could be expected on the basis of association between ornamental brightness and size. However, as has been recently shown by Shawkey et al. (2007) higher levels of brightness may by caused by the feather-degradating activity of keratinolytic bacteria on the feathers. The activity of keratinolytic bacteria could be regulated by the preening ability which in turn may be associated with individual's quality. Therefore plumage brightness could indeed signal to females a low level of male's health. This result is not unique. For instance Dufva and Allander (1995) found that great tits with more intense colouration have also higher heterophil levels in the peripheral blood. Elevated H/L ratio is in this species known to be a sign of stress or bad health (Ots et al. 1998).

But, brightness is also positively correlated with the PHA skin swelling response which is commonly assumed to be a reliable indicator of individual immune capacity. The interaction between ornamental colour and immune capacity was shown in many species (e.g. Birkhead et al. 2006; Nolan et al. 2006). Nevertheless, usually this association concerns hue or saturation. To answer why the situation is different in scarlet rosefinch we have to consider the immunological nature of this inflammatory process. PHA-induced swelling is referred to be mediated by basophilic hypersensitivity reaction (Corrier & Deloach 1990; Parmentier et al. 1993; Scott & Siopes 1994; Maxwell & Robertson 1995). It was proved in several experiments in birds that after the injection of PHA into the wing web within several hours there is a massive infiltration of granulocytes and especially basophils into the afflicted tissue (McCorkle et al. 1980; Martin et al. 2006). From this point of view there would be no surprise if birds with high levels of basophils in peripheral blood (those with higher plumage brightness) would be able to aggregate more basophils into the swelling and mount a stronger response to PHA. Indeed, after a closer analysis we have shown that basophil ratio predicts from almost 30% the PHA responsiveness and that inclusion of the basophil counts into the model completely erases the predictive potential of ornamental brightness. The resulting model containing basophil counts, size and heterozygosity was able to explain as much as 55% of the total variance in the swelling responses. PHA is undoubtedly not the natural substance to which the basophil immunological component is directed. Therefore it seems that the results of the PHA skin-swelling test are in this case more indicative to a current disease than to the ability to fight any potential parasites. This view is also supported by the fact that on the contrary to other studies we did not find any positive relationship between condition and immunity measured by the PHA skin test (Parn et al. 2005; Smith et al. 2007) or between hue and saturation and this immunity measurement (Faivre et al. 2003a; Faivre et al. 2003b; McGraw & Ardia 2003; Alonso-Alvarez et al. 2004; Birkhead et al. 2006). It is, however, still rather doubtful by which mechanism the PHA responsiveness is associated with the multilocus heterozygosity. The result of ours is in fact the same as that of Hawley et al. (2005) in house finch. In principle, there are two possible explanations. First, heterozygosity might be positively associated by its general beneficial influence with the immune function (see e.g. Hawley et al. 2005; Reid et al. 2005) creating an additive effect on swelling both in high-basophil and low-basophil birds. This might be for instance in some cases due to the inbreeding effect which was found to lower the PHA responses (Reid et al. 2007). The other possibility is that only several loci that are in linkage disequilibrium with some key loci participating in immune defence regulation effect the response. Fossøy (2007) found that exactly this is the case in bluethroat nestlings and it is probable that this situation is quite common (see Pemberton 2004). Even in such a case a similar additive effect of heterozygosity on swelling would be observed. Some of the microsatellite loci used in this study were the same as those used by Hawley et al. (2005) and so the positive association of heterozygosity to PHA-induced swelling could be in both these studies caused by the same loci. Unfortunately we are unable to decide between these two possibilities as our sample size is too small to enable us statistical testing of the effect of individual microsatellite loci on the swelling responsiveness to PHA.

Our results support the hypothesis of multiple signal components. There was no correlation between the three components of male ornamental colour. Thus scarlet rosefinch females might be able to gain a different type of information from these independent signals. According to hue, they might be able to assess the food-gaining ability of the potential partner while on the basis of plumage brightness they could judge the male long-lasting condition costs and general health, including the infection status of some diseases. Our data suggest that especially larger birds might face elevated stress. This claim is also indicated by the fact that in our population larger birds develop drabber plumage. The data then support the hypothesis H.A.1. on health-indicatory function of the male ornamental plumage colouration in scarlet rosefinch. These results are consistent with the former findings of Schnitzer (2003) who provided evidence that the colour of scarlet rosefinch males correlates with the male reproductive success. Hence the sexual advertising might play an important role in host-parasite coevolution (Møller et al. 2000). We failed to support the hypothesis H.A.2. because we did not find any direct influence of genome-wide heterozygosity on ornament elaboration. However, we present evidence suggesting the interaction between heterozygosity and the magnitude of immune responsiveness to PHA (H.B.2.) as well as supporting the relationship between ornament and immunoresponsiveness (H.B.1.).

VIII FACTORS DETERMINING HEALTH AND IMMUNE RESPONSIVENESS IN SCARLET ROSEFINCH BROODS

VIII.1 Introduction

The individual's health is a basic factor influencing the lifetime reproductive success and thus also the overall individual fitness. This is particularly true for nestlings in which health and a general condition is the matter of survival until future reproduction in adulthood. Of special importance for the individual's health is withal the ability to overcome diseases caused by parasite attacks. In animals the anti-parasite resistance is controlled by the immune system which is especially in birds highly developed. However, even in spite of the general efficiency of the avian immunity there is a considerable variation in the individual capability to resist various infections. As the result we observe a remarkable variation in the health-related traits and survival. These traits are often related one to another and therefore factors influencing the condition (measured as weight or size) alter to some degree also hematological and immunological parameters as well as the survivorship itself (Gonzalez et al. 1999; Hõrak et al. 2000; Johnsen et al. 2000; Tella et al. 2000; Tella et al. 2001; Møller & Petrie 2002; Kleven & Lifjeld 2004; Westneat et al. 2004). But what are the factors creating the variability in parasite resistance and ergo determining the health? It is widely accepted that phenotype is codetermined by genotype and environmental factors. Analogously we can expect that the condition-related traits composing general health (such as weight, size, haematological parameters, immune function and parasite burden) as well as survival will be governed partly by genetical factors and partly by the environment.

Evolutionary the most important part of the striking among-individual variance in anti-parasite resistance and survivorship that we observe in natural animal populations is due to the differences in genetical background among individuals. At least some of the genotype traits associated with the disease resistance are heritable and so can be focused by the sexual selection as proposed by the model of parasite-mediated sexual selection (Hamilton & Zuk 1982). Especially recently our understanding of these genetical factors influencing health has been much promoted. With the development of molecular immunology and immunogenetics voluminous evidence has been earned showing us the function of various alleles of particular immunomodulative molecules in anti-parasite defence. In most vertebrate taxa the key role of the MHC was proved and also in birds it was found that MHC genotype determines the responsiveness of the humoral, T cell as well as the phagocytic immunity component and that these components are quantitatively independent (Lamont & Smyth 1984; Cheng & Lamont 1988; Zhou & Lamont 2003; Buchholz et al. 2004). Moreover, even in wild species the association between certain MHC loci and the resistance to specific parasites has been recently proved (Westerdahl et al. 2005; Bonneaud et al. 2006; see also Piertney & Oliver 2006). Similar relationship is likely to exist between particular parasite prevalence and MHC allele numbers (Westerdahl et al. 2005; Vinkler et al. 2007). It was also confirmed by (von Schantz et al. 1997) that MHC genotype may indicate the individual ability of following survival. Although also some other loci are supposed to be immunologically important (Acevedo-Whitehouse & Cunningham 2006) our knowledge on their structure and function of their products is still very limited in birds and therefore no studies in ecological immunology focused on their associations with the health and anti-parasite resistance in wildly living species. However, there are also several general genetical factors that may influence the quality of anti-parasite defence and from various reasons cannot be adopted by the 'good gene' mechanism of sexual selection. Among these is often considered the genome-wide heterozygosity, which is non-heritable, and individual's sex, which is above the selection forces directing mate choice evolution. Indeed, nowadays there is some evidence linking the individual's heterozygosity in neutral markers with survivorship (Foerster et al. 2003) or parasite resistance (Hawley et al. 2005). Moreover, it was proved that heterozygosity may be also positively related to the quality of mounting cellular immune response (Hawley et al. 2005; Fossøy 2007), which might be, however, due to the linkage disequilibrium of the neutral markers with some immunologically important loci (Fossøy 2007). Finally, individual's sex is governing many aspects of the immune system function (e.g. PHA hypersensitivity responsiveness, Cheng & Lamont 1988; Tschirren et al. 2003; Jovani et al. 2004; McGraw & Ardia 2005; antibody production, Hasselquist et al. 1999; Saino et al. 2003a; McGraw & Ardia 2005; or activity of phagocytes, Cheng & Lamont 1988) and also influences haematological parameters (Lucas & Jamroz 1961; Zuk 1996; Ots et al. 1998; Hõrak et al. 1998), parasite loads (Ots et al. 1998) and parasite susceptibility (Tschirren et al. 2003).

Although genetical factors are of great importance to the activity of the immune system their final effect is highly dependent on the environmental factors. Rearing environment influences nestling health especially through parental care and food abundance. Food abundance unsurprisingly determines both the nestling's growth (Birkhead et al. 1999) and the general health and immune capacity (Klasing 1998; Hoi-Leitner et al. 2001; Lifjeld et al. 2002; Saino et al. 2003b). Moreover, even the diet composition with respect to the presence of some particular nutrients may influence the immune function (Lochmiller et al. 1993; Dietert et al. 1994; Parmentier et al. 1997; Birkhead et al. 1999; Gonzalez et al. 1999; El Abasy et al. 2002; Fenoglio et al. 2002b; Cheng et al. 2004) and so determine the survival (Birkhead et al. 1999). The immune capacity of parents and their nestlings determines the present parasite burden which in turn significantly influences their condition and immunity. In many studies it was proved that individuals fighting against current infections are able to develop a weaker response to experimental immunological challenges (Christe et al. 2000; Navarro et al. 2003; Westneat et al. 2004) and show altered haematological parameters (Ots & Hõrak 1998; Davis et al. 2004; Campbell & Ellis 2007). Among environmental factors influencing health and immunity we can also count stress which besides to all that mentioned above may result from the social environment and artificial handling conditions (Dietert et al. 1994; Ewenson et al. 2001; Ewenson et al. 2003; El Lethey et al. 2003; Hawley et al. 2006). El Lethey et al. (2003) and Ewenson et al. (2001) provided evidence that especially elevated H/L ratio in peripheral blood is a good indicator of stress.

In the study described in this section we aspired to investigate the general factors influencing the condition, health and immune responsiveness to PHA in scarlet rosefinch nestlings. The attention was paid especially to the relationship between parental genotypes and their phenotypical traits on one side and health-related traits in the nestlings on the other. In our study population it has been recently shown that females do not choose their social partners with respect to their MHC genotype (although this is probably not true for the extra-pair mates Promerová 2007). Therefore here we investigated another important genetical characteristic – parental multilocus relatedness.

We have proposed following hypotheses:

C. parental traits are predictive to health and immunological traits of their young

H.C.1. General health and immune responsiveness in young are associated with social male's ornamentation. Results supporting this hypothesis would be in concordance with the 'Good genes' mechanism of sexual selection rather than with the Complementarity hypothesis because male colouration might predict the genetic quality of the father.

H.C.2. Immune responsiveness and health in nestlings are determined by parental relatedness. Supportive evidence for this hypothesis would suggest the importance of the 'Complementary genes' mechanism of the sexual selection.

VIII.2 Methods

The relationships between parental traits and condition, health and immune responsiveness were investigated in the period 2004-2007 on the study site northwards from the Želnava village (N 48°49', E13°56', area about 61 ha; in figure VI.1-2 labeled as 'Želnava'). Each year the nests of scarlet rosefinches were systematically searched and located during a several day period in first half of June. In each nest the number of eggs was recorded. In those cases where the clutch was obviously incomplete another visit after few days was done to enable estimating the probable day of hatching. Afterwards each nest was visited two days before the expected date of hatching and since then one control was performed per day (in the afternoon) until all young hatched. After finding a young in the nest the date and time of the control were noted and the nestling was individually marked with a permanent marker (specific black pattern on the legs) to enable later discrimination. During the control all nestlings were weighted with Pesola gauge (Pesola, MicroLine 20010, 10g, d=0.1g). Each visit to the nest took no longer than ten minutes and all the marking and weighting was performed on a place about 20 m distant from the nest to minimise the risk of attracting a predator. For the same reason any approaching to the nest was done by the method described by Martin and Geupel (1993). After all offspring hatched no more visits of the nest occurred until the first nestling was 7 days old. On the day 7 post hatch (individually for each nestling according to their hatching date) each nestling was ringed with a darken steel ring, weighted with Pesola gauge (MicroLine 20060, 60g, d=0.5g), the tarsus length was measured using digital calliper Kinex, type 6040.2 (accuracy 0.01mm), blood smear was prepared and the injection of PHA was treated into the left wing (as described below). Later, on day 8 post hatch the swelling response of each nestling was measured and the weight and tarsus length were noted. Again all approaches to the nest were done carefully, time consuming manipulations with the nestlings were performed on places distant from the nest and no nestling spent more than 30 minutes out of the nest. All measurements were always done by one and the same person only (MV). Later in the laboratory the blood smears were stained according to Pappenheim (see paragraph VII.2.2) and the leucocyte differential count was recorded. Out of these counts the heterophil / lymphocyte ratio (H/L) was estimated which is a reliable measure for health indication (Ots et al. 1998). No other haematological examinations were done because of the necessity of avoiding a possible harmful effect of larger blood volume losses in the nestlings. The presence of EPP in the nest was examined as described in paragraph IX.2.1.

VIII.2.1 Measurements of the immune response in nestlings

All measurements of the immune responsiveness in nestlings were performed during the afternoon control of the nests (from 15 p.m. to19 p.m.; for application of the PHA skin-swelling test in the afternoon see e.g. Navarro et al. 2003 or Marzal et al. 2005). The PHA treatment (again according to Smits et al. 1999) was performed strictly in the age of 7 days post hatch, i.e. individually for each young in the nest. The spot in the centre of the patagium was marked and the thickness of the wing web was measured three times independently. Then the injection of 0.20 mg PHA-P (Sigma, product No. L-8754) dissolved in 20.0 µl PBS (Sigma, product No. D-5652) was treated by Hamilton syringe (type 710 RN) into the marked place of skin. Thereafter the time of the injection was noted and the young one was placed back into the nest. After 24 hours ± 1 hour the magnitude of the swelling in the marked spot was measured by three independent measurements. For the measurements of the thickness of the wing web a digital calliper Kinex, type 6040.2 (accuracy 0.01mm) was used. The closely fit of the measuring arm on the skin surface was controlled visually to ensure no deformation of the tissue by the pressure of the calliper. All measurements were always done by one and the same person only (MV). The repeatability of the three successive measurements was considerably high (r=0.87). For further analyses the mean of these three measurements was used. After performing all necessary measurements the young was placed back into its nest and after the measurements of the youngest nestling the nest was never disturbed again.

VIII.2.2 Collecting data on adult parent birds in the course of breeding

In the course of the second half of nestling-feeding period (individually in each nest) in each year of the study all parental birds were captured into the mist nets in the vicinity of their nests. The birds were immediately transported in a fabric bag to a suitable place nearby, far enough to let the second parental bird feed their offspring without any stress. The manipulation with adult birds was the same as with the pre-breeding males (for details see the paragraph VII.2), only no immunity measurements were performed in order to preserve the natural course of the nesting. In short, the captured individual was weighted and then the metrical measurements were taken. Then the sex was noted. In the case of males also the ornament was documented by digital photographs and a rectrix was taken. Thereafter a blood sample for further genetical analyses was collected and a blood smear was prepared. After collecting all necessary data the bird was ringed with one standard steel ring and a combination of three colourful plastic rings (AVINET) for later visual identification. Afterwards the animal was immediately released. Again all measurements were done by one and the same person only (MV). All following processing of the obtained samples was identical to that described in paragraph VII.2 for the pre-breeding adult males. Parental relatedness was calculated by Radka Poláková on the basis of multilocus microsatellite data described in paragraph VII.2.6.

VIII.2.3 Determination of the sex of nestlings

Isolation of the DNA from the blood samples was performed by DNeasy®Tissue Kit (50) (Qiagen, No. 69504). DNA Analysis of the nestlings' sex was performed by J. Schnitzer with the acquisition of the DNA test described in Griffiths et al. (1998). The test is based on two CHD genes that are located on the avian sex chromosomes: CHD-W gene on the W chromosome and CHD-Z gene on the Z chromosome which occurs in both sexes. Using PCR with a single set of primers parts of both genes including introns (whose lengths usually differ) were amplified. Therefore, when examining PCR products on gel a single band characterised males while two bands females.

VIII.3 Results

VIII.3.1 Nests

In the period 2004-2007 76 scarlet rosefinch nests were found in the study habitat (16, 22, 23 and 15 respectively). In total these nests contained 347 eggs (71, 98, 98 and 75 respectively) varying from 1 to 6 eggs per clutch (median 5, var. 0.938). Of these eggs 261 hatched into the hatchlings (60, 77, 62 and 62 respectively). Nest predation on eggs was the cause of a hatching failure in 6 cases (in total 30 eggs; 0, 2, 4 and 0 depredated nests in successive years respectively). As other sources of the hatching failure were identified inundation during floods (in 5 nests, 13 eggs) and embryo mortality of an unknown reason (that, however, occurred only in 2 known individual cases). Much more important was a parental fertility failure or an early developmental failure manifested as embryo lacking observed in 19 individual cases (8 nests: 0, 5, 7 and 7 eggs from 2004 to 2007 respectively) which in 2 nests caused a complete nesting failure. In remaining 27 eggs belonging to 17 nests the cause of the hatching failure was not identified while in these cases the egg disappeared before it could be examined. In total the nesting failure on eggs was 24.78 % (15.49%, 21.43%, 39.81% and 17.33% in the successive years respectively).

Out of the 261 hatched nestlings only 201 successfully survived until the age of 8 days post hatch. 43 young ones originating from 10 nests (0, 2, 5 and 3 respectively) were depredated and remaining 17 nestlings from 12 nests (3, 4, 4 and 1 respectively) died for other reasons such as coldness or starvation. The scarlet rosefinch nestling survival in the period 2004-2007 was assessed on 77.01% (95.00%, 83.56%, 54.84% and 79.03% respectively).

VIII.3.2 Haematology in nestlings

I examined the differential leucocyte counts in 129 nestlings from the period 2004-2007. The description is summarized in table VIII.3.2-1. No blood parasites were detected.

Table VIII.3.2-1:
Differential leucocyte count in scarlet rosefinch nestlings (n=129).

Cell type	Range (%)	Mean ± conf. limits	St. deviation	Median
Lymphocytes	5 - 41	16.23 ± 1.26	7.27	15
Immature cells	0 - 19	2.54 ± 0.51	2.96	2
Heterophils	1 - 26	9.67 ± 0.82	4.74	9
Basophils	8 - 83	53.45 ± 1.77	10.15	53
Eosinophils	2 - 34	16.26 ± 1.17	6.69	16
Monocytes	0 - 11	2.10 ± 0.32	1.8	2

We investigated whether the mean brood haematological characteristics are related to any traits that are supposed to influence offspring fitness. First we proposed a generalized linear model in which H/L ratio was expected to be dependent on parental relatedness, male ornamentation (hue, saturation and brightness), date of hatching, mean initial weight of the nestlings and on the presence or absence of extra-pair youngs in the brood. This model was tested on a subset of 25 nests in which all required parameters were known. The resulting minimal adequate model comprised of nestling mean initial weight (p=0.090) and male breast plumage saturation (p=0.049; for detail information see table VIII.3.2-2) and explained 27.13% of the total variability in H/L ratio among broods. Then we examined a similar model on the same sample for the basophil count. In this case we failed to find any significant relationship between the predicted parameters and the amount of basophils in peripheral blood of nestlings (the closest interaction was to social male's ornamental plumage saturation with p=0.151).

Table VIII.3.2-2:
Minimal adequate model for the mean brood H/L ratio, n=25, df=2/24, F=4.09, p=0.030.

Variable	Slope ± SE	df	F	p
H/L				
Mean initial weight	- 0.12 ± 0.069	1/23	3.14	0.090
Social father's saturation	-0.012 ± 0.006	1/23	4.34	0.049

Figure VIII.3.2-1:
Association of the mean brood H/L ratio and male ornamental saturation, n=25. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.

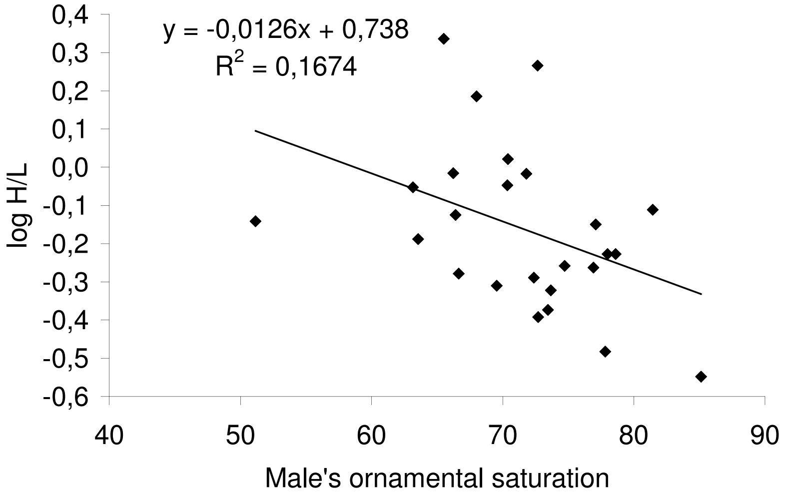
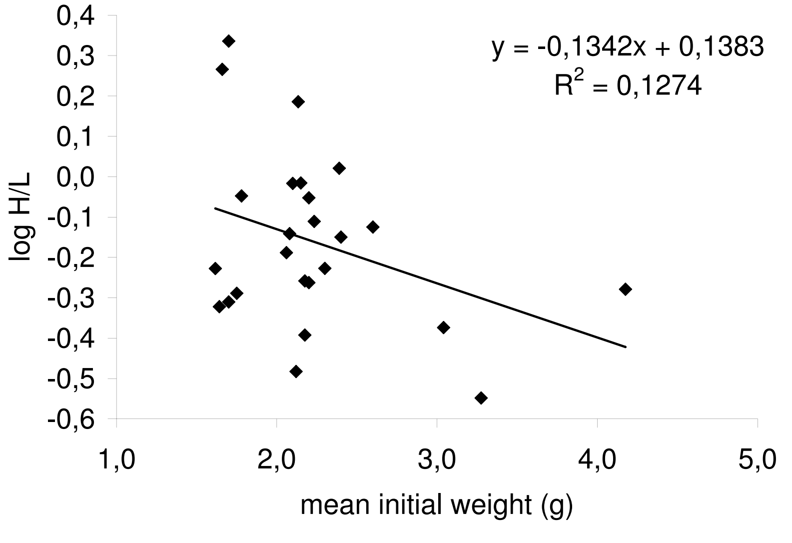


Figure VIII.3.2-2:
Relationship between the mean brood H/L ratio and mean brood initial weight, n=25. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.



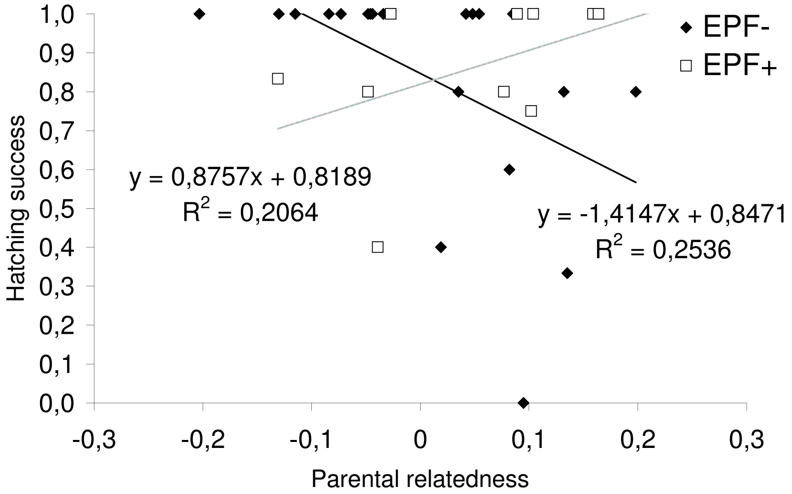
VIII.3.3 Effect of parental traits on hatching success in their nests

In a subset of 30 nests we tested whether parental relatedness, EPF or social male ornamentation is predictive to the hatching success (two-way interactions were included). We found no relationship between any of these variables and the hatching success but there was significant association between the hatching success and the interaction parental relatedness:EPF (p=0.014; for detail information see table VIII.3.3-1).There was no difference between broods that contained EPY and those that did not with respect to parental relatedness (t=-0.96, p=0.344).

Table VIII.3.3-1:
Minimal adequate model for the hatching success, n=30, df=3/29, F=3.11, p=0.043.

Variable	Slope ± SE	df	F	p
Hatching success				
Parental relatedness	3.115 ± 2.966	1/28	1.90	0.179
EPF	0.263 ± 0.296	1/28	0.13	0.716
Parental relatedness: EPF	- 9.745 ± 2.966	1/27	6.88	0.014

Figure VIII.3.3-1:
Association of the hatching success and parental relatedness with respect to the presence of EPFs, n=30.



VIII.3.4 Brood characteristic correlates of the cell-mediated immune responsiveness in nestlings

The data on mean PHA responses were obtained in total from 52 nests (period 2004-2007). However only for 26 of them we had all necessary additional data to include them into the overall analysis. In this subset the mean brood PHA-induced swelling response ranged between 0.42 mm and 1.30mm (variance 0.06). Using a generalized linear model we examined whether parental relatedness, hatching date, mean hatching weight, mean growth rate (difference between initial weight and weight in the age of 8 days post hatch), male ornamentation or the presence of EPP in the nest may predict the mean brood immune responsiveness to PHA. We found that the social male ornamental colour (hue and saturation) correlates with the mean nestling immunoresponsiveness (p=0.022 and p=0.001 respectively; see table VIII.3.4-1 for the minimal adequate model). This model explained 38.97% of the total variance in this immunological trait. From the graph on figure VIII.3.4-1 it is obvious that in the case of hue the significant negative association between hue and mean brood PHA responsiveness is predominantly due to one single out-lying point. However, exclusion of this individual from the dataset would be incorrect as the orange plumage colouration is within the range of variability spectra naturally occurring in our study population. Nevertheless, even we if exclude this individual out of the database there is still a slightly non-significant tendency for the association of mean brood PHA responsiveness with social father's hue (p=0.077) while for saturation is this relationship again highly significant (p= 0.001).

Table VIII.3.4-1:
Minimal adequate model for the mean brood immune response to PHA, n=26, df=2/25, F=7.34, p=0.003.

Variable	Slope ± SE	df	F	p
PHA				
Social father's hue	- 0.025 ± 0.010	1/24	6.06	0.022
Social father's saturation	0.023 ± 0.006	1/24	13.73	0.001

Figure VIII.3.4-1:
Relationship between the mean brood PHA responsiveness and social male ornament hue, n=26. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.

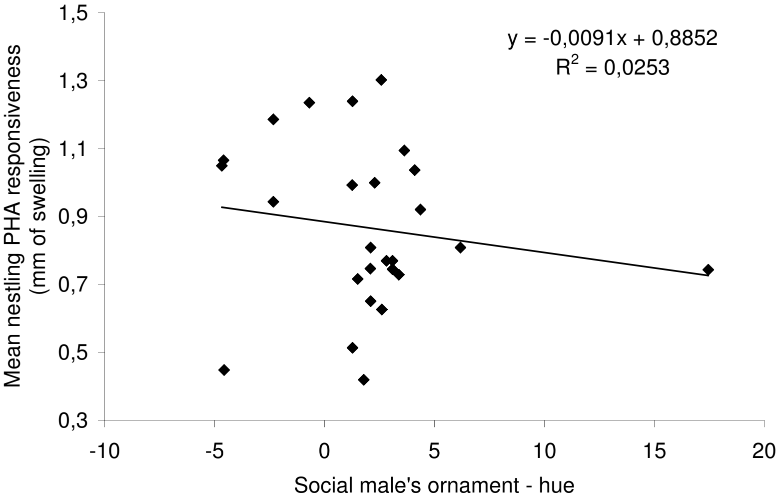
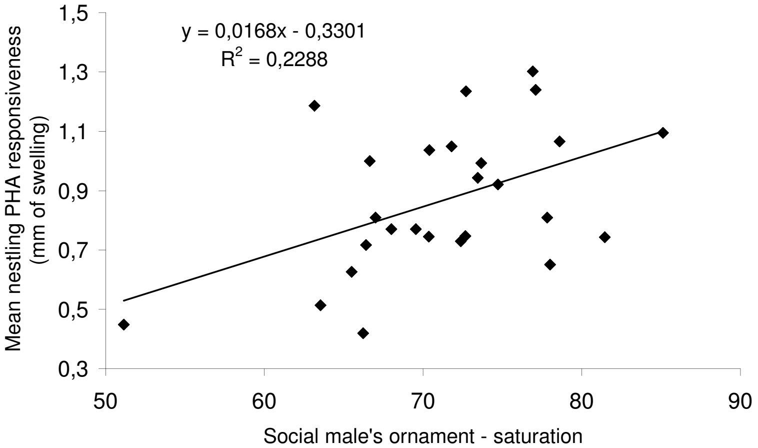


Figure VIII.3.4-2:
Association between the mean brood PHA responsiveness and social male ornament saturation, n=26. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.



VIII.3.5 Individual characteristic correlates of the cell-mediated immune responsiveness

In the period 2004-2007 we collected the data on PHA skin-swelling response from 192 young. Out of these we had all required information for further model testing in 123 nestlings. This subset was used for the analysis. The PHA swelling response varied from 0.14 mm to 2.44 mm (variance 0.11). To investigate the individual-related variables influencing the magnitude of the PHA-induced skin swelling response we set up a model in which the dependence of the PHA response on growth, sex, hatching order, hatching date and interaction of hatching order and hatching date, H/L ratio, basophil count, hatching weight, weight in the age of 8 days, size in the age of 8 days and extra-pair paternity was tested. The brood identity was used as a random effect. The resultant minimal adequate model is summarized in table VIII.3.5-1. The only significant effect was found for the growth rate (p=0.027). However, there was also a tendency for slightly non-significant influence of sex and interaction of hatching order and hatching date.

Table VIII.3.5-1:
Minimal adequate model for the individual nestling immune response to PHA, n=124, df=5, Chi=12.765, p=0.0257.

Variable	Estimate ± SE	df	Chi	p
PHA				
Intercept	0.503 ± 0.319			
Growth	0.039 ± 0.018	1	4.865	0.027
Hatching order	- 0.025 ± 0.060	1	0.173	0.678
Hatching date	- 0.012 ± 0.010	1	1.589	0.208
Sex	- 0.012 ± 0.010	1	3.478	0.062
Hatching order: hatching date	0.030 ± 0.016	1	3.489	0.062

Figure VIII.3.5-1:
Association between the nestling PHA responsiveness and growth rate, n=124. In this graph the growth rate is shown as a mean day increase in body weight (g per day). The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.

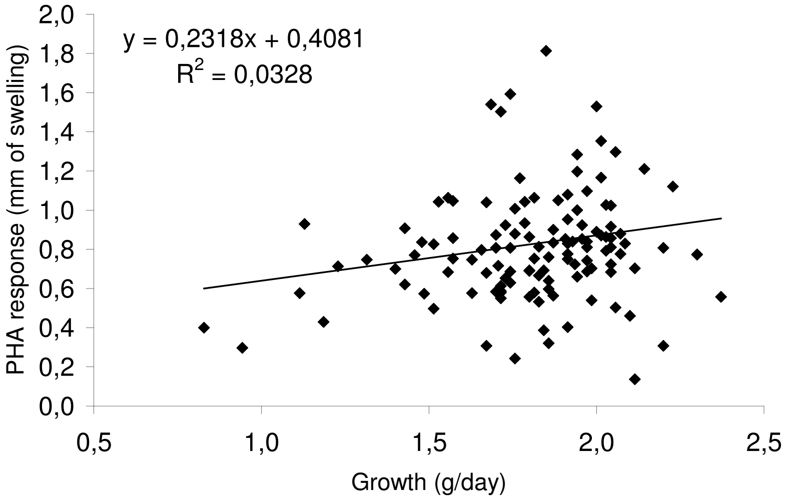


Figure VIII.3.5-2:
Difference between female (F) and male (M) nestlings in PHA responsiveness, n=124. The boxes indicate quartiles with highlighted median.

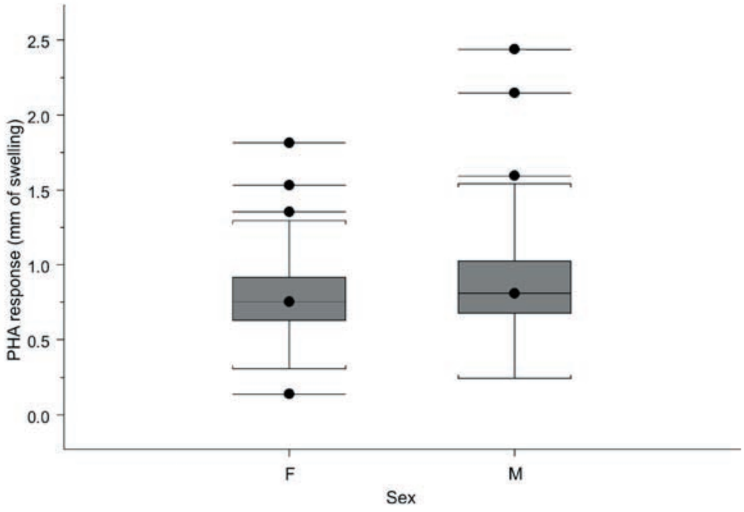
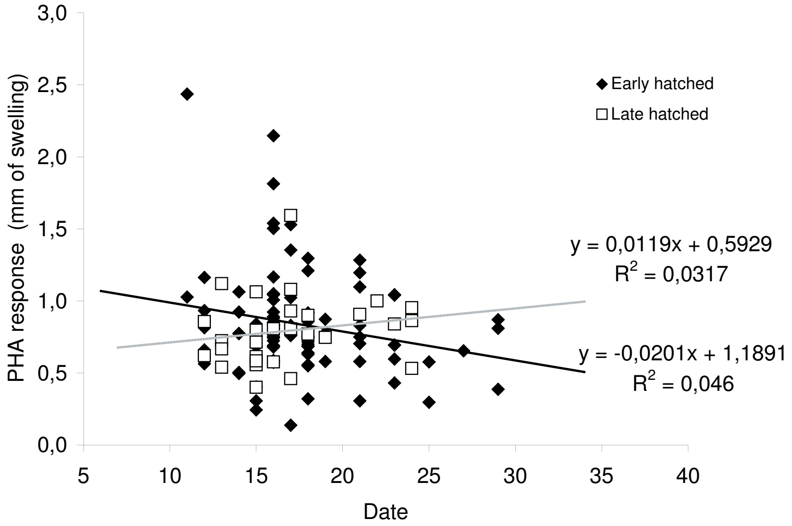


Figure VIII.3.5-3:
Relationship between the nestling PHA responsiveness and the hatching date with respect to the hatching order, n=124. The figure is based on linear regression while a generalized linear model was adopted for the statistical analysis.



VIII.4 Discussion

In our scarlet rosefinch study population we found that the parental relatedness is associated with hatching success when controlling on EPP. There was no effect of the genetic similarity between parents on offspring health or immune function. Nevertheless we found correlation between social male's ornamentation on one side and condition and cell-mediated immunoresponsiveness of nestlings in his brood on the other side. Hue and saturation of the male's ornament were able to predict the swelling response in young from almost 40%. The social father's plumage saturation correlated negatively with mean nestling H/L ratio in his brood. On the individual level we found that the immune response was significantly linked to growth rate. No effect of extra-pair paternity on condition or immunity was detected.

The hatching failure represented in our study population a significant cause of lowered parental reproductive success. In a great majority of cases the unhatched eggs did not show any signs of development. As referred by Birkhead et al. (1995) in house sparrow only about 1% of laid eggs are infertile. Thus a great proportion of hatching failures is probably because of embryo mortality, potentially as an effect of parental genome incomplementarity. In several species it was proved that particularly parental relatedness decreases hatching success of young (Kempenaers et al. 1996) or their survival rates (Foerster et al. 2003; Oh & Badyaev 2006, but see Edly-Wright et al. 2007). Although we were unable to investigate whether parental relatedness decreases nestling and fledgling survival, we did so in the case of hatching success. When considering the effect of EPP we found clear influence of parental genetic similarity on the proportion of unhatched eggs in their nests. The hatching success decreased significantly with the parental relatedness unless female improved the viability of her eggs by EPF. However, it does not seem that females seek for EPFs to enhance the hatching success of their eggs because the distribution of EPP was random with respect to within-social-pair relatedness. Therefore these results do not support the hypothesis of the extra-pair mate choice based on genetic complementarity. Rather it seems that the EPCs could represent random strategy of offspring viability insurance for the case that parental relatedness is too high. However, as our data are only correlative in their nature further experimental testing of this hypothesis is needed. As the distribution of parental relatedness among pairs was Gaussian it does not seem that females discriminate genetically related males in their social mate choice.

Although it is difficult to follow the pair bond forming in wildly living birds it is possible to estimate the female mate choice criteria on the basis of potential fitness benefits that females gain from the appropriate choice. In scarlet rosefinch Schnitzer (2003) showed that the intensity of male's ornamentation indicates his food provisioning to nestlings. Hence females gain direct benefits from mating with males with more intense colouration. Here we tried to investigate whether they also gain any other fitness benefits such as enhanced health or better immunity of their nestlings. We have shown that male ornamentation may indicate both health and cell-mediated immune responsiveness in offspring. Thus our results support more the 'Good genes' model than the genetic complementarity hypothesis although other explanations are also possible (see below).

Contrary to Fossøy (2007) who has shown in bluethroats that EPP increases the mean PHA response in the broods and that the magnitude of PHA response is influenced by the parental relatedness, we did not find any effect of parental genetic similarity or EPP on the PHA responsiveness in nestlings. Similar results as ours have been recently described also by Edly-Wright et al. (2007) who found in house sparrows no association between parental genetic similarity and either immunocompetence or condition and hatching success in young.

On individual level we found that the immune responsiveness to PHA is directed by the growth rate. This result is identical to that obtained earlier by Hõrak et al. (2000) in great tit nestlings. Therefore it seems that both H/L ratio and PHA responsiveness reflect nestling's nutritional condition. It is therefore possible that the male's ornamentation is reflected by the nestlings' health and immune function due to differences in parental food provisioning activity which enhances the mean nestling condition within broods of more intensely coloured males. It is well-established that male colouration may reflect the amount of potential parental care provided by the male (Hill 1991) and this is also true in our scarlet rosefinch population Schnitzer (2003). Linville et al. (1998) moreover showed that the male plumage colouration is in some species positively related to the female feeding effort. It is also a well-known fact that the immune function development, including PHA responsiveness, as well as the growth rate are co-determined by the quality of diet (Lochmiller et al. 1993; Birkhead et al. 1999). Saino et al. (1997) and Hoi-Leitner et al. (2001) provided evidence that parental food provisioning rate and the amount of food per nestling are two basic components determining the magnitude of the PHA swelling response in young. Moreover, the mechanism of linkage between male ornamentation and PHA responsiveness in offspring proposed above is in concordance with the recent finding of Pitala et al. (2007) who have reported that the PHA response might be in fact a non-heritable trait. Very similar results are presented also by Tella et al. (2000) from their study on American kestrels (*Falco sparverius*) and by Christe et al. (2000) from house martin (*Delichon urbica*). The PHA skin-swelling test is, indeed, a good indicator of the nestling's condition and its future survival (see Moreno et al. 2005) but the involvement of any 'good genes' in the imunoresponsivness to PHA is unclear.

However, our results are interesting in one more way. In young that hatched later in the hatching order we detected a tendency to mount greater immune responsiveness than in the earlier hatched young but only when they hatched later in the breeding season. This reminds the situation observed by Saino et al. (2001) in swallows (*Hirundo rustica*). The likely explanation to this state is that young in a poorer condition (late hatched young in suboptimal conditions at the end of the breeding season) have to invest more into the non-adaptive immune responsiveness to survive. This might represent a response of the youngest nestling to the mechanism proposed by Christe et al. (1998) as 'Testy chick hypothesis' which predicts the weakest young to be a preferential target for parasites (but see Christe et al. 1998 for opposite results than those found in this study).

We found a clear association between social male parental ornamentation and general health and immune responsiveness of his young (supporting H.C.1.). Our data, however, suggest the possible importance of 'Good parent' model (see Hoelzer 1989) in rosefinch sexual selection although the validity of 'Good genes' mechanism mediated by honest signalling as predicted by the Viability indicator hypothesis may be equally right. However, our results do not support the 'Good genes' hypothesis with respect to EPP as we failed to detect any effect of the presence of EPP on nestling immunoresponsiveness. Nevertheless, it is possible that the effect of EPP is not general and that it appears only in the direct comparison of EPY with WPY from the same brood. Although we found that higher genetic relatedness of parents may lower the hatching success in young we did not find any supportive evidence for the 'Complementary genes' hypothesis per se (H.C.2.).

IX EXTRA-PAIR PATERNITY AND OFFSPRING FITNESS COMPONENTS: DO FEMALES BENEFIT FROM EPFS?

IX.1 Introduction

Offspring quality is one of the most important components determining the individual's fitness. It is therefore commonly assumed that the choice of a sexual partner is non-random with espect either to the genetical quality or to the genetic complementarity of the potential mate (see chapters IV.1.1 and IV.1.2). However, in some cases female's choice may be inappropriate (although this is undoubtedly a simplification, let us presume that the pairing results from the female mating strategy predominantly). This may come for instance due to the lack of suitable mates available or due to hasty onset on breeding resulting from a trade-off between the careful social partner choice and the requirement of early nesting. It was proposed that both these conditions (the limited mate sample and the rash choice) may be particularly common in some long-distant migrating monogamous passerines with a short breeding season (Albrecht et al. 2007). The female of a socially monogamous species that is paired to a male of inferior genetical quality has then in principle only two possibilities: either remain faithful (which leads to fitness penalisation) or get engaged in extra-pair mating to improve the fitness potential of her offspring (which may be, however, punished by her social partner, see Arnqvist & Kirkpatrick 2005). As already mentioned in chapter IV the second case is perhaps quite common in birds (Griffith et al. 2002) although the evolutionary causes for the observed EPP rates are still rather uncertain.

If we presuppose that the EPP is beneficial from the point of enhanced offspring fitness it is necessary to ask what these benefits are and how they are gained. The answer to neither of these questions is clear under the contemporary knowledge. As fitness benefits in young are usually studied the components of individual survivorship such as hatching success, nestlings' body condition, growth, nestlings' survival, immunity related traits (mostly PHA skin swelling responsiveness and antibody production), haematological health indicators, parasite loads or later fledgling's survivorship. Only in very few cases direct benefits from EPP were reported (see e.g. Gray 1997) and therefore genetical factors are believed to play a more important role. However, when concerning the effect of parental genotypes it is equally valid to suppose that the mate choice is based on individual genetical traits as well as that it is governed by endeavour to achieve complementarity of the male and female genomes. Parental complementarity is supposed to represent a comparable source of genetical benefits to offspring as 'good genes' per se (Brown 1997).

In some species the evidence indicates the presence of the 'Good genes' mechanism behind the extra-pair mate choice. For example Sheldon et al. (1997) found in collared flycatcher (*Ficedulla albicollis*) by comparisons of maternal half-sibs in mixed paternity broods that the sire genotype, which is reflected by his ornamentation, is crucial for the determination of the offspring condition. Similar results are reported also by Kempenaers et al. (1992; 1997) from their blue tit studies in which they found that in broods with mixed paternity EPY, that are preferentially fathered by older and larger males exhibiting longer strophes in their song and better annual survival, survived significantly better than WPY.

On the other hand, there is also a wealth of evidence for the hypothesis linking the extra-pair mate choice with 'complementary genes' benefits (understood usually as an advantage of the most heterozygous genomes in offspring). As found by Blomqvist et al. (2002) in three shorebird species (western sandpipers *Calidris mauri*, common sandpipers *Actitis hypoleuca* and Kentish plovers

Charadrius alexandrinus) the social pair EPP levels are in all these species strongly positively related to genetic similarity between partners within the social bond. Interestingly, Foerster et al. (2003) revealed in blue tits (*Parus caeruleus*, the same species as studied by Kempenaers et al. (1997) that females may increase through EPPs the average heterozygosity of their progeny, and these authors also showed that more heterozygous individuals have increased survival chances, more elaborated male secondary sexual trait (crown colour) and higher reproductive success. In house finch mating between unrelated partners brings strong fitness benefits through increased fledglings' survival (Oh & Badyaev 2006). Moreover, Lindstedt et al. (2007) have recently found in house finches that the occurrence of EPFs closely covaries with genetic relatedness of mates and that offspring arisen from genetically dissimilar mating tended to be more resistant to novel pathogen infections.

Besides to what has been already mentioned it is also possible that even within one single species several forms of mating strategies may coexist (see e.g. Oh & Badyaev 2006 who found in house finch that the genome complementarity based mate choice alternates with the mate choice based on ornamental traits).

Albrecht et al. (2007) have recently shown that scarlet rosefinch males significantly differ in their ability to enhance their reproductive success though extra-pair mating and that extra-pair mating success is related to the paternity in the male's own nest. This suggests that females might choose the genetical fathers of their offspring (within-pair as well as extra-pair) according to some trait which might indicate the enhanced offspring fitness. Indeed, Schnitzer (2003) found that the paternity in rosefinches is correlated with the male's ornamentation. This species may be supposed to be exposed to high risk of inappropriate social mating due to the very short time which is available for the females to choose among the males displaying on the nesting site (Björklund 1990) and thus females may use EPP to improve their former pairing mistakes. This view is supported also by recent findings that the paternity looses in social males' nests are related to males' low levels of MHC diversity (Poláková et al. 2007b). Moreover, females prefer for extra-pair mating the males with higher genome-wide heterozygosity than is their own one and also that of their social partner (Poláková et al. 2007b). In this part of the study we hence aimed to map potential fitness related benefits in nestlings resulting from EPP in scarlet rosefinch. As our sample size was limited we decided for two analyses. We examined whether there is any difference between EPY and WPY in their multilocus heterozygosity as the positive result would indicate us the involvement of genome complementarity mechanisms. Then we compared the immune responsiveness in EPY with their WPY half-sibs to test if there are any immune-related benefits arising from EPP. However, first of all we analysed whether the male quality-related traits predict the parentage in his own nest.

We proposed following hypotheses:

D. There are differences in parental traits between broods that contain EPY and those that do not

H.D.1. Social male's ornamentation predicts EPP.

H.D.2. Social male's condition traits predict the occurrence of EPP.
The positive result when testing both of these hypotheses would support the 'Good genes' hypothesis as males of higher quality would loose less paternity in their nests and then could have higher total reproductive success than lower quality males.

E. Extra-pair young differ in their immune responsiveness and heterozygosity from their maternal within-pair half-sibs.

H.E.1. The maternal half-sibs differ in the average level of their heterozygosity. The support of this hypothesis would indicate the validity of 'Complementary genes' hypothesis.

H.E.2. There is a significant difference in PHA skin-swelling responsiveness between EPY and WPY from the same brood. The higher immune responsiveness in EPY would represent evidence for indirect female benefits from EPP.

IX.2 Methods

The data analysed in this part of the study were obtained together with the data analysed in the section VIII. In this section the data concerning nests with extra pair paternity are reanalysed with the emphasis on the comparison of maternal half-sibs differing in their sires. Everything else being identical I describe here only the additional assay of parentage performed on the collected samples.

IX.2.1 Identification of parentage

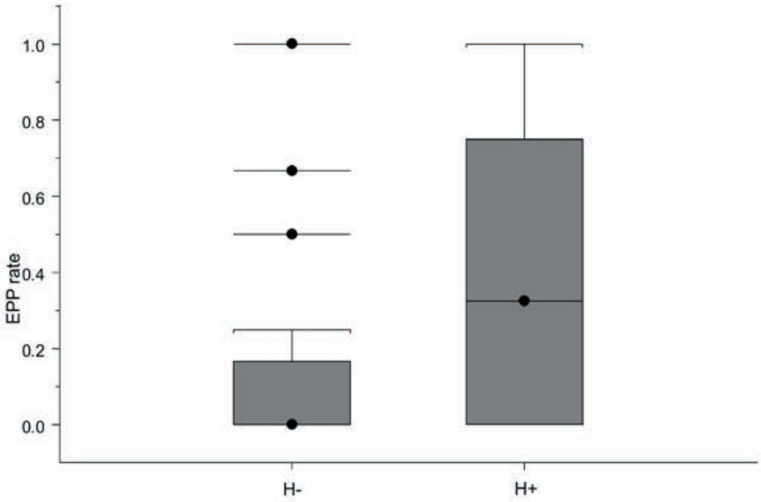
Parentage identification was performed by J. Schnitzer as a part of his dissertation thesis. Here the methods used for the identification of parentage are described only briefly (for detailes see Albrecht et al. 2007). The blood samples collected in the field (see section VII.2 and VIII.2) and stored in the absolute spirit were dried and DNA was extracted and purified using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany). The parentage analysis was conducted using 3 microsatellite loci (LOX2, LOX7, and LOX8) originally developed for the Scottish crossbill (*Loxia scotica*; Piertney et al. 1998). These loci were proved to be extremely variable in rosefinch. Each adult individual possessed a unique genotype. Therefore although only 3 loci were used, the total exclusionary power exceeded 99% for both parents. The offspring's paternal alleles were subsequently compared with the alleles of its putative father (the female's social partner). If the alleles did not match at 2 or 3 loci, the putative father was excluded as a sire and the young was considered as EPY.

XI.3 Results

XI.3.1 Social male's traits and EPP occurrence

The correlation between the male's ornamental and the condition-related traits on one side and the differences among broods in the frequency of EPY on the other side was tested by a generalized linear model. As independent variables were used male's ornamental hue, saturation, brightness, *Haemoproteus* occurrence, avian pox occurrence, H/L ratio, basophil count, size and mass. There was no association between male ornamentation and EPP frequency in his nest. We detected only a non-significant tendency for males infected with *Haemoproteus* to be more often cuckolded (n=33, slope=1.303±SE 0.473, df=1/32, F=3.24, p=0.081).

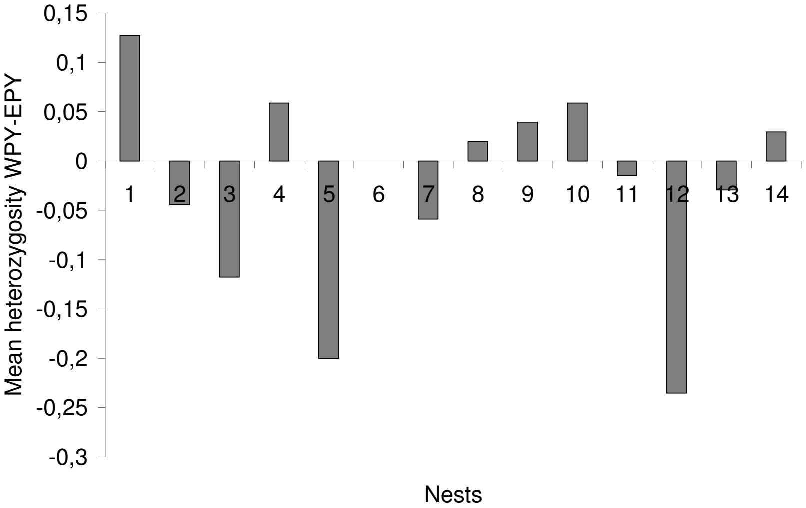
Figure IX.3.1-1:
Difference between *Haemoproteus* negative (H-) and *Haemoproteus* positive (H+) males in the proportion of extra-pair paternity in their nests, n=33. The boxes indicate quartiles with highlighted median.



IX.3.2 Extra-pair paternity and offspring heterozygosity

In a subset of 14 nests with mixed paternity from the period 2003-2006 we used the Paired t-test to compare the mean EPY and WPY multilocus heterozygosity. We found no difference between EPY and WPY from the same nests with respect to their mean heterozygosity (n=14, df=13, t=0.97, p=0.349).

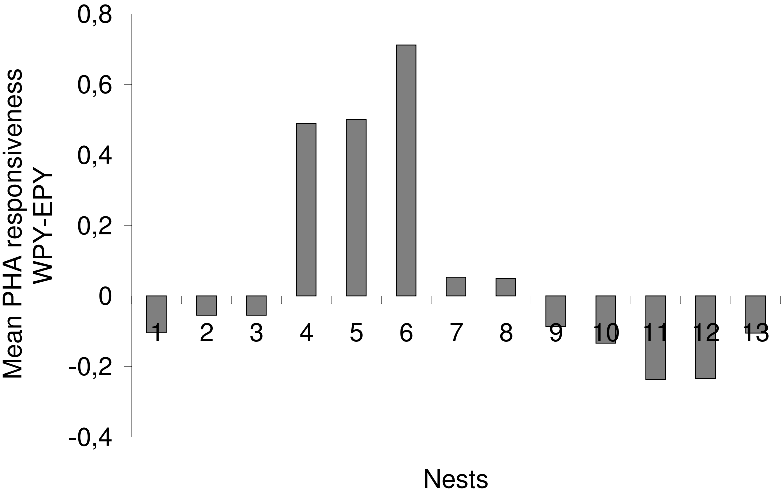
Figure IX.3.2-1:
Among-nest variation in the difference in mean multilocus heterozygosity between maternal half-sibs differing in paternity, n=14.



IX.3.3 Extra-pair paternity and cell-mediated immunity

To investigate the association between EPP and immune responses to PHA we compared the mean responses of EPY and WPY from the same broods in a subset of 13 nests from the period 2004-2007. Again, we found no difference between EPY and WPY in their mean cell-mediated immune responsiveness to PHA (n=13, df=12, t=-0.72, p=0.483).

Figure IX.3.3-1:
Among-nest variation in the difference in mean PHA responsiveness between maternal half-sibs differing in paternity, n=13.



IX.4 Discussion

Our results do not indicate that in the investigated years females chose males for extra-pair mating to improve their former inappropriate social mating with respect to male ornamental colouration. Less extensively ornamented males were not cuckolded more often than expected on the basis of mating by chance. There was only a weak tendency for *Haemoproteus* parasitized males to be cheated with greater frequency. Moreover, we found no difference in the multilocus heterozygosity between EPY and WPY. Neither we have detected any association between EPP and immune responsiveness to PHA. It is possible that these negative results were caused by the low sample size. However, there was even no indication of any tendency for the EPY to be generally more heterozygous or 'immunocompetent'. Hence, our data support neither the hypothesis that females improve the heterozygosity of their young by EPC nor that they choose the extra-pair males to gain for their offspring benefits related to immunity. These results must be, nevertheless, treated with some caution and considered only as preliminary because of the low sample size available for the comparison of heterozygosity and immune swelling response between EPY and WPY within the same brood.

Currently there is a considerable amount of studies supporting the relationship between the male's ornamental plumage colour and the probability of extra-pair paternity in his nest (Yezerinac & Weatherhead 1997; Møller & Ninni 1998; Krebs et al. 2004). On the contrary, many studies failed to find such a relationship (Strohbach et al. 1998; Cordero et al. 1999; Kraaijeveld et al. 2004). This lack of association was found among others also in house finch, a species closely related to scarlet rosefinch (Hill et al. 1994). In rosefinch it was previously shown that there may be correlation between the male ornament intensity and the probability of cuckoldry (Schnitzer 2003). Contrary to this result here we did not find any such relationship. To explain this discrepancy we may consider several hypotheses. It is possible that the variability among males available for the mate choice differs significantly among the years which may result in our impaired ability to detect the differences in the male's colouration in some years. Indeed, Albrecht (unpublished data) compared the variability in ornamental colouration of males in our study population from 2001 to 2007 and found that there was a significant decreasing trend. Although these differences in variability of the male ornamentation among the years might have arisen by various ways, an interesting possibility is that the observed situation results from cycles in host-parasite competition as described by Hamilton and Zuk (1982). It is obvious that what is important to the female choice is not the total variation among males throughout all the years but rather the actual available variation within one breeding attempt (Bateson & Healy 2005). Therefore further analyses should be based on a sample originating from more years than available for this study and scoring the male colouration as an order of the particular male among the other males within the same breeding season. Highly useful would be also the direct comparison of the cuckolded males with their cuckolders. Unfortunately either in this case our data did not enable us such an examination. It is possible that the time-related differences in extra-pair paternity linkage to the male ornamentation are due to the involvement of the context-dependent female choice. It was found by Oh & Badyaev (2006) that in house finch females may switch their mate choice with respect to available male genetic variability from the ornamental-based mate choice to the relatedness-based mate choice and vice versa. It is possible that a similar principle takes place also in scarlet rosefinch. If so, then females could choose their mates on the basis of ornamentation in the periods with high interindividual variability in the secondary sexual traits and on the basis of other traits (such as MHC haplotype complementarity selected by post-copulatory choice) in the years with low variability. Again this supposition has to be further tested.

Our results however do not indicate that females were choosing their extra-pair mates to improve the genome-wide heterozygosity of their offspring. Contrary to Foerster et al. (2003) we did not find any tendency for EPY to be more heterozygous. Our results are rather consistent with those of Schmoll et al. (2005) who found in coal tits (*Parus ater*) no difference in genetic similarity between females and their social partners on one side and between females and their extra-pair partners on the other. Similarly, neither Bouwman et al. (2006) nor Kleven and Lifjeld (2005) detected in reed buntings (*Emberiza schoeniclus*) any effect of relatedness on within-pair or extra-pair mating. Moreover, as in our case, Kleven and Lifjeld (2005) failed to find any differences in heterozygosity between the maternal-half sibs and principally analogous results were obtained also by Kempenaers et al. (1996) in blue tits. Surprisingly, Ratti et al. (1995) even found in pied flycatcher (*Ficedula hypoleuca*) a higher incidence of EPP in genetically more dissimilar breeding pairs. Thus, heterozygosity across the whole genome may not be so important in non-inbred populations (although in inbred populations it might be of great importance and might be responsible for differences in immunity, Reid et al. 2007). However, although we did not find any differences in multilocus heterozygosity by direct comparison of EPY and WPY it would be incorrect to conclude that there is no genome complementarity benefit from EPP in scarlet rosefinch. This lack of correlation may be easily explained by the insufficient number of the used microsatellite loci for accurate estimate of the true genome-wide heterozygosity (Pemberton 2004). Another possibility is that the complementarity in some particular loci (such as MHC) is much more important than the genome-wide heterozygosity per se. This view is supported by the finding that in our study population the social male MHC diversity is negatively related to the occurrence of EPP in the male's nest (Poláková et al. 2007b). At the same time it was however shown that females cheat on their social males with more heterozygous extra-pair males (Poláková et al. 2007b). It is therefore even possible that the extra-pair sexual selection in scarlet rosefinch is driven by the Genetic diversity model.

Contrary to the findings of some other studies (e.g. Johnsen et al. 2000; Garvin et al. 2006; Fossøy 2007) we did not detect any differences between EPY and WPY originating from the same brood with respect to the cell-mediated immune response to PHA. It is possible that this result is caused by only limited sample size which did not allow us to detect minute differences in this trait and differences in the variance which might be also important. However, similar negative results were obtained also in some other similar studies (e.g. Kleven & Lifjeld 2004; Kleven et al. 2006). It is possible that although the PHA skin swelling test is widely used measurement of 'immunocompetence' it may not reliably reflect the immune capacity of the examined individual and its anti-parasite resistance potential (Cheng & Lamont 1988; Ewenson et al. 2001; Blount et al. 2003; Saks et al. 2006; Kennedy & Nager 2006; Owen & Clayton 2007). Hence we might fail to focus on a trait important to the nestling fitness and therefore we missed the quality differences between EPY and WPY. For instance Charmantier et al. (2004) found that although the EPY and WPY did not differ in any condition-related measurement still there was significantly higher fledging survival in EPY. Moreover, even if the test was appropriate the association between EPP and immune responsiveness might be context-dependent (see Garvin et al. 2006; O'Brien & Dawson 2007). On the basis of our data we cannot exclude the possibility that in our study population the fitness differences between EPY and WPY emerge later in the ontogeny or under special environmental conditions. As pointed out by Kokko (2001), the individual fitness comprises of many components that might be undetectable by the used approaches and so negative results when testing immunity differences between EPY and WPY do not mean the evidence for falsification of the 'Good genes' hypothesis. The same is true for the 'Complementary genes' hypothesis as well. Another possibility is that the extra-pair female choice is maintained by the sexual selection without higher offspring survival on the basis of enhanced offspring attractiveness (Kokko et al. 2002).

We failed to support any of the proposed hypotheses (H.D.1.-2., H.E.1.-2.). Therefore we are unable to rule out the possibility suggested by Arnqvist & Kirkpatrick (2005) that the extra-pair mating may not be beneficial for the female fitness. In rosefinch the fitness benefits from EPP for males are well-established (Albrecht et al. 2007), however, similar benefits to females remain unclear and speculative.

X CONCLUSION

In the study encompassed in this thesis my colleagues and I have investigated three principal aspects of the sexual selection in scarlet rosefinch: 1) the reliability of male ornamentation as an indicator of individual's quality; 2) the relationships between parental traits and offspring health indicators; 3) the effect of paternity on offspring heterozygosity and immune responsiveness in mixed-paternity broods.

Our data suggest that male ornamentation is a reliable indicator of individual's health that may be used by females in their mate choice. Genome-wide heterozygosity however does not seem to be reflected by male's ornamentation. From the point of a female mate choice it is of special importance that social father's ornamentation is a good predictor of the health of the nestlings in his nest. This result is much more congruent with the 'Good genes' hypothesis than with the Hypothesis of 'complementary genes' at least when concerning the female choice of a social partner. However, as there was no effect of EPP on the nestlings' health and the swelling response to PHA was related especially to the growth rate (which presumably depends on the amount of available food), it is possible that the relationship between male's ornamentation and young quality is not genetical but that it is rather due to the rearing environment in the nest. As referred by Schnitzer (2003) in our study population the male colouration indicates the amount of parental care. Therefore it seems that the young quality is predominantly determined by the primary female choice of her social partner. The mechanism of the mate choice might be that described by 'Good parent' hypothesis (Hoelzer 1989). Concerning the extra-pair mate choice we did not prove any fitness benefits arising from EPFs. The EPY were neither more heterozygous than their maternal half-sibs nor they mounted stronger immune responses. Therefore we failed to support either the 'Good genes' hypothesis or the 'Complementary genes' hypothesis. Nevertheless, on the basis of these results it is not possible to reject the validity of any of them. It is possible that we focused on relatively unimportant fitness predicting indicators and genetic complementarity markers. The genetic complementarity could be based on some other genetic features than is the genome-wide heterozygosity. Much more important might be some key loci. Moreover, although it does not seem that parental relatedness plays in mating any important role, it is possible that a limited amount of microsatellite loci that were available could have caused wrong estimates of among-individual relatedness. Although this eventuality cannot be ruled out, it is important to note that our sample of microsatellite loci was much higher than used in other similar studies (usually up to 10). Furthermore, our finding that the parental relatedness predicts the hatching success indicates the validity of our estimates.

Contrary to Schnitzer (2003) we did not find any relationship between male ornamentation and his paternity within the brood he rears. It seems that this lack of association might be connected with among-years changes in the variance in male ornamentation. This might suggest the existence of two interesting phenomena within our rosefinch population. First, the female extra-pair choice could be context-dependent, given by the actual variability in males. Second, the variability in male ornamentation may be associated with host-parasite evolution. Nonetheless, these speculations highly require further testing.

Interestingly, we have shown in adult males that the magnitude of the cell-mediated hypersensitivity response to PHA in the skin-swelling test comprises of two basic components. It is determined by some genetical factors (possibly genome-wide heterozygosity) but probably more importantly also by the total amount of granulocytes (i.e. especially basophils) available in the blood stream. This result throws a new light on our understanding of the meaning of this test. As elevated levels of granulocytes in peripheral blood usually indicate stress or disease it seems that if the information on the haematological state of the individual is not added into the analysis it is impossible to judge whether the higher magnitude of the response indicates high or low quality of the individual. Surprisingly, we did not find any effect of basophil counts on the magnitude of PHA skin-swelling response in nestlings. This might be caused by lower variance in basophil proportion in peripheral blood in nestlings when compared to adult males. However, as the immune system of young 7 days post hatch is still developing it is also possible that the mechanism of the inflammatory response and thus also cell infiltration in young differs from that in adults (see also results of Uller et al. 2006). Hence in young the responsiveness to PHA may not be directly related to the basophil presence in blood but rather to the general condition.

To conclude, our results are not fully consistent either with 'Complementary genes' hypothesis or with the 'Good genes' hypothesis, although there is more supportive evidence for the 'Good genes' hypothesis. Future studies are necessary to resolve the question of potential female benefits from EPP in this species. As emphasised by the current study the research effort should be devoted especially to testing the heritability of the male quality indicators on a larger sample of broods with mixed paternity or by cross-fostering experiments and to comparisons of the results obtained in successive years to map the factors responsible for the prospective context-dependent extra-pair female mate choice. Broaden should be also the range of investigated genetical and phenotypical traits that could influence the survivorship or pairing ability of the young. As scarlet rosefinch is from many aspects a valuable model species exciting results can be expected in these future studies.

Table X-1:
Main results of this study and their congruence with the 'Good genes' hypothesis and 'Complementary genes' hypothesis.

Result	'Good genes'	'Complementary genes'
Male ornamentation reflects health	+	-
Male ornamentation does not reflect heterozygosity	+	-
Male ornamentation predicts quality of nestlings in his nest	+	-
Parental relatedness does not predict nestlings' quality	+	-
No association between male ornamentation and EPP occurrence	-	+
No difference between EPY and WPY in immune responsiveness	-	-
No difference between EPY and WPY in heterozygosity	+	-

XI ACKNOWLEDGEMENT

First of all I wish to acknowledge my supervisor Tomáš Albrecht for his wide-ranging support and especially for his initial idea of studying the immune system involvement in the sexual selection in birds. I give my special thanks also to Jan Schnitzer for his attentive assistance with the field data collection and providing me with the data on the ornament colouration, sex of the nestlings and paternity that he analysed. For some of the data used here I am much obliged also to Radka Poláková (individual heterozygosity and parental genetical relatedness) and Jan Votýpka (blood parasites in 2004 and 2005). I am grateful to Pavel Munclinger that he kindly accepted me to his office, helped us in the field and gave me some useful advice. I would also like to appreciate the help from Martin Lundák, Lubor Červa, Jaroslav Jelínek, František Zicha, Pavel Jaška and Hana Mrkvičková with the field work. I would like to thank both Doc. Vladimír Holáň for his original comments to the research and Professor Drahoslav Pravda for his remarks to the haematological methods adopted in this study. My gratitude belongs last but not least to my mother and Martina Pokorná for their comments to the manuscript and all my family for their lifelong support and attentive patience. This work was supported by Grant Agency of Charles University (project 191/2004/B-Bio), Grant Agency of the Academy of Sciences of the Czech Republic (project IAA600930608) and Grant Agency of the Czech Republic (project 206/06/0851).

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