

---

# Tail colouration and disease susceptibility in the greenfinch

---

Marion Foley-Fisher

---

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science and the Diploma of Imperial College  
London

## Contents

Acknowledgements .....	6
Abstract .....	7
1. Introduction .....	8
1.1 Colour, disease and honest signalling .....	8
1.2 Issue .....	9
1.3 Aims and Predictions.....	9
2. Background .....	11
2.1.1 Sexual Selection and honest signals .....	11
2.1.2 Hamilton and Zuk hypothesis.....	11
2.1.3 Honest signalling.....	12
2.2.1 Structure and acquisition of carotenoids .....	13
2.2.2 Functions of carotenoids within the body.....	15
2.2.3 Metabolism of carotenoids for pigments.....	17
2.2.4 Deposition of carotenoids for colour .....	17
2.2.5 Honest signalling and trade off with carotenoid pigments .....	18
2.2.6 Key studies in bird plumage .....	18
2.3.1 Infectious diseases .....	19
2.3.2 Emergent infectious diseases.....	20
2.3.3 Salmonella.....	21
2.3.4 Trichomonas .....	21
2.4 Garden bird project.....	22
2.5.1 Greenfinches .....	22
2.5.2 Greenfinches, infectious disease and sexual selection.....	23
2.6 How to measure colour.....	24

3. Method .....	26
3.1 Source of Samples .....	26
3.2 Post-mortem examination .....	27
3.3 Colour analysis of feathers .....	27
3.4 Data analysis .....	29
4. Results.....	31
5. Discussion .....	39
References .....	45
Figures and Tables .....	51

DECLARATION OF OWN WORK

I declare that this thesis "Tail colouration and disease susceptibility in the greenfinch" is entirely my own work and that where material could be construed as the work of others, it is fully cited and referenced, and/or with appropriate acknowledgement given.

Signature .....

Name of student .....

(please print)

Name of Supervisor .....

**AUTHORISATION TO HOLD ELECTRONIC COPY OF MSc THESIS**

Thesis title: Tail colouration and disease susceptibility in the greenfinch

Author: Marion Foley-Fisher

I hereby assign to Imperial College London, Division of Biology, the right to hold an electronic copy of the thesis identified above and any supplemental

tables, illustrations, appendices or other information submitted therewith (the "thesis") in all forms and media, effective when and if the thesis is accepted by the College. This authorisation includes the right to adapt the presentation of the thesis abstract for use in conjunction with computer systems and programs, including reproduction or publication in machine-readable form and incorporation in electronic retrieval systems. Access to the thesis will be limited to Conservation Science MSc teaching staff and students and this can be extended to other College staff and students by permission of the Conservation Science MSc Course Directors/Examiners Board.

Signed:

Name printed:

Date:

### **Acknowledgements**

I would first like to thank my main supervisor, John Ewen for offering me the opportunity to carry out this research project and for his dedication and enthusiasm. His input was invaluable to this project and I am very grateful for his guidance. Secondly I would like to thank Marcus Rowcliffe, especially for his help with the statistical analyses and writing up. Finally big thanks to Becki Lawson for giving me permission to use the GBHi database and for giving up so much of her time to help with the data collection, including carrying out many of the post-mortem examinations and allowing me use of the post-mortem room at the Institute of Zoology.

**Abstract**

Carotenoid-based feather colouration in birds has been hypothesised to signal individual genetic resistance to parasites. A trade off exists between using carotenoids for immune function and detoxification and investing them in plumage colour. It is predicted that brighter birds will have a greater resistance to disease. This project tests this hypothesis on the greenfinch, *Carduelis chloris*, by comparing the plumage colour of birds who have died from infectious diseases and those that have died from trauma. I found that adult male greenfinches were more likely to have died from a disease than younger birds or females. This may have been due to the fact that adult males are dominant at feeding stations, where there is a high risk of disease contamination. Adult male birds that had died from the infectious disease, trichomonas, were duller than those who had not died from trichomonas, thereby supporting the hypothesis. Adult male birds that had died from intestinal parasites or salmonella were actually brighter than birds that had not died from these diseases. Ornamental plumage in greenfinches is used as a social status signal and therefore the brighter birds are more dominant. Therefore these findings also support the dominance theory suggesting that socially dominant birds are at more exposed to infectious diseases at feeding stations, and therefore are more likely to die from an infectious disease.

## **1. Introduction**

### **1.1 Colour, disease and honest signalling**

There has been a large body of research suggesting that carotenoid-based ornamental plumage colouration is a sexually selected trait in birds (reviewed in Hill, 1991; Møller, 1988; Hill & McGraw 2006). Females prefer to mate with males who have larger, brighter ornaments (Lindström and Lundström, 2000) and these ornaments are assumed to be an honest signal of genetic fitness (Zahavi, 1975). In greenfinches (*Carduelis chloris*) and common red polls (*Carduelis flammea*), as well as many other garden birds, ornamental plumage is carotenoid-based (McGraw, 2006 in Hill, 2006 ch.5). Carotenoids cannot be synthesised in the body and therefore have to be consumed in the diet (reviewed in Surai, 2003). They can either be deposited in feathers for colouration, or they can be utilised by the immune system and used as antioxidants (Constanti and Møller, 2008; Surai, 2003). Using carotenoids for colouration can be detrimental to survival, as fewer carotenoids are then available if an immune response needs to be mounted during a disease outbreak. There is a risk involved in investing carotenoids in feathers and therefore only the healthiest birds, with the highest disease resistance can afford to be colourful (Møller, 1990). Females may therefore choose brighter males based on their carotenoids signalling as they are better able to resist infectious disease. If disease resistance and feather colouration are heritable then the trait can be passed on to their offspring (Hamilton and Zuk, 1982). More directly females may simply avoid mating with males that are at a higher risk of disease, to reduce their chances of contamination.

This theory has been supported by studies showing that carotenoid-based ornamental plumage colouration is often positively associated with the ability to resist infectious diseases. For example, Horak et al (2004) found that greenfinches that were infected with the intestinal parasite, coccidia, had duller ornamental plumage. Hill and Farmer (2004) inoculated house finches (*Carpodacus mexicanus*) with a novel pathogen, *Mycoplasma gallicepticum* and found that redder birds were able to clear the disease much quicker than dull birds.

Other studies have found the opposite relationship, for example Van Oort and Dawson (2005) found that in common redpolls brighter males were more likely to have died from salmonella than dull males. Similarly, in a study by Horak et al. (2001), older male great tits (*Parus major*) that were infected with haemoparasites had higher values of hue than their uninfected counterparts. Van Oort

and Dawson (2005) explain this reverse relationship by dominance, whereby brighter, dominant birds have greater access to feeding stations, where there is increased risk of contamination from sick birds. Infectious diseases are easily transmitted through water and food and therefore feeding stations and bird baths provide the ideal conditions for rapid disease transmission.

## 1.2 Issue

British garden birds are becoming increasingly at risk from infectious diseases and emergent infectious diseases. As the public take more of an interest in providing food on bird tables for garden birds so there has been an increase in the spread of diseases (Becki Lawson, unpublished data). The emerging infectious disease, trichomonas, is spread through faecal transmission and the infectious disease salmonella is spread through saliva (GBHi, trichomonas advice sheet; GBHi, salmonella advice sheet). A high density of birds around feeding stations facilitates the spread of these diseases through large amounts of faeces and salival contamination of food (GBHi, Best practice guidelines).

As a result of these concerns, the Garden Bird Health Initiative was set up in 2003 to promote research into the diseases affecting birds and identify those that were most at risk. To reduce the disease risks at feeding stations they have been advocating disinfecting feeding stations and having several stations to reduce the density of birds at any one site (GBHi, Best practice guidelines). As part of the monitoring network set up by the GBHi, volunteers who find dead birds are requested to send them to the GBHi for post-mortem analysis. Greenfinches (*Carduelis chloris*) make up 34% of the birds that have been sent in suggesting that they are a high risk species (Becki Lawson, unpublished data). As greenfinches are known to use their carotenoid-plumage for sexual signalling, and they are at high risk from infectious diseases and emerging infectious diseases, they are ideal candidates for my investigation. The purpose of this study is to see if there is a link between disease status and carotenoid-based plumage colouration in greenfinches, in order to better understand colour as a predictor of susceptibility to infectious disease.

## 1.3 Aims and Predictions

1) To assess whether the likelihood of a greenfinch dying from trichomonas, salmonella or intestinal parasites is related to its sex, age or the habitat it lives in. I predict that:

- Adult male greenfinches will be more likely to die from trichomonas, salmonella or intestinal parasites than females or younger birds. Adult males are more likely to be exposed to pathogens at feeding stations due to their dominant social status and therefore have an increased risk of infection.
- Greenfinches found in rural areas will be less likely to die from an infectious disease compared to greenfinches found in urban areas. This is because there are more feeding stations in urban areas and therefore the risk of catching an infectious disease is higher than in rural areas. In rural areas, birds do not have to rely on feeding stations as a food source and can feed in less dense groups reducing the chances of disease transmission.

2) To ascertain whether yellow chroma and brightness, and UV chroma and brightness, are predictors of an adult male greenfinches susceptibility to mortality from infectious disease, specifically trichomonas, salmonella or intestinal parasites. I predict that:

- Birds that are more likely to die from infectious disease outbreaks will have lower values of brightness and chroma in the carotenoid-based ornaments of their tail feathers. Brighter birds have been able to invest their carotenoids in ornamental colouration as they are better able to survive during infectious disease outbreaks.

## **2. Background**

### **2.1.1 Sexual Selection and honest signals**

Sexual selection is the evolution of traits that arise from variation among individuals and seemingly have no benefit to survival. These traits do however affect success in competition for mates and fertilisations (Andersson, 1994). There are two major theories in sexual selection; the “runaway” hypothesis (Fisher, 1915, reviewed in Andersson, 1994) and the “good genes” hypothesis (Zahavi, 1975). In the “runaway” hypothesis, females develop a preference for a certain trait in males that is unrelated to their fitness and mate with these males. Their offspring will therefore inherit the trait as well as the preference for the trait. The “good genes” hypothesis says that male display indicates some form of fitness that is controlled by genes. By mating with a male who has highly developed ornamentation, a female ensures that her offspring inherit these good genes. Hamilton and Zuk (1982) postulated that sex traits were reliable indicators of an individual’s ability to resist disease. Females could decipher which males were affected by diseases by the extent of the expression of their ornamentation. Therefore female mate choice would benefit the offspring by increasing their chances of survival (Andersson, 1994). Over the last decade, the good genes hypothesis has received growing attention and recent work primarily focuses on the associated idea that sexually selected ornamentation is an honest signal of the quality of the bearer.

### **2.1.2 Hamilton and Zuk hypothesis**

Hamilton and Zuk (1982) offered one route by which sexually selected ornaments may be honest indicators of quality by hypothesizing that host resistance and parasite genotype were cyclical. A host could have the genotype, H or h and parasites could be P or p. H is resistant to p, but not P and h is resistant to P, but not p. The hypothesis dictates that if p is the most prevalent parasite to affect the next generation, then females should choose a male who has the genotype H, so that their offspring will be more likely to be resistant to the common parasite, p. If this happens, then it is more beneficial for the parasites to have the genotype P and thus this becomes the more common parasite in the population. In turn, the advantage of being H falls and the population of hosts will tend towards to the genotype h. Cycles may be of varying length and several cycles can occur at

once. Hamilton and Zuk (1982) proposed that genetic disease resistance is signalled through secondary sexual traits, so a bird who has greater expression of plumage ornamentation will have an above average inherited fitness. Hamilton and Zuk (1982) also found that species which were most prone to virulent parasites had the most developed sexual traits.

Evidence is accumulating that supports the ideas of sexually selected ornamental traits being used in mate selection, that they are heritable and also provide information about the quality of the bearer. For example in 1988, Møller found that female swallows (*Hirundo rustica*), preferred to mate with males that had longer tail feathers, a sexually selected trait. As swallows are monogamous, males need to make sure they are chosen by females first so that the breeding season can be elongated, allowing time for two clutches in one year. The most attractive males also benefit from being chosen early by females who are able to double-clutch. A study by Hill (1991), found that females house finches (*Carpodacus mexicanus*) preferred to mate with males who had brighter plumage and were more colourful. Brighter males were also found to be more attentive to nests and had better overwinter survival, which could therefore have been what the females were selecting for. Sons who had bright fathers, went on to develop bright plumage themselves, demonstrating that this is a heritable trait. In a meta-analysis of 40 different species (including birds, insects, spiders and fish) Jennions et al. (2001) found that males with larger ornaments, weapons or body size had better chances of survival.

### **2.1.3 Honest signalling**

For a signal to be reliable it must be costly to produce or maintain. Costly production or maintenance therefore leads to honest signalling such as is required for under the Hamilton and Zuk (1982) hypothesis. Honesty, however, does not have to rely on parasite mediated selection to operate. For example, having bright plumage can be costly as it makes an individual more conspicuous to predators (Andersson, 1994). Evans and Thomas (1992) found that the long tail of a male scarlet-tufted malachite sunbird (*Nectarinia johnstoni*) has detrimental aerodynamic effects on flight and therefore the ability to catch insects. Costs of plumage signals are also dependant on the types of pigments utilised in feather colouration. There are three main broad types of pigment. These are melanins which produce black and brown colouring, carotenoids that produce yellow and red colouring and structural pigmentation which produces greens and blues (Hill, 2006). Carotenoid-

based plumage signals are particularly good candidates for honest signalling because of the difficulty in their acquisition and in the multiple and competing roles they perform (see below).

After melanins, carotenoids are the most common type of pigment in feather plumage colouration (McGraw, 2006 in Hill, 2006 chp.5). Greenfinches (*Carduelis chloris*) for example use carotenoids to colour their ornamental feathers (Lindström and Lundström, 2000). As we shall see later in this section, investing carotenoids in feather colouration means that there are less available for other important functions within the body. Carotenoids have roles in the immune system (Constanti and Møller, 2008; Surai, 2003) and therefore birds who have weaker immune systems need to use carotenoids to help fight future infections. It is argued that male birds who have brighter plumage are therefore signalling to females that they are good at resisting disease (Hamilton and Zuk, 1982) making carotenoid pigmented ornaments condition-dependent (honest) signals.

### 2.2.1 Structure and acquisition of carotenoids

Carotenoids are 40-carbon tetraterpenoid molecules, made up of a series of isoprene residues whose sequence is reversed at the centre (McGraw, 2006 in Hill, 2006 ch.5). Below is a structural diagram of the carotenoid lutein, which is a common pigment in birds.

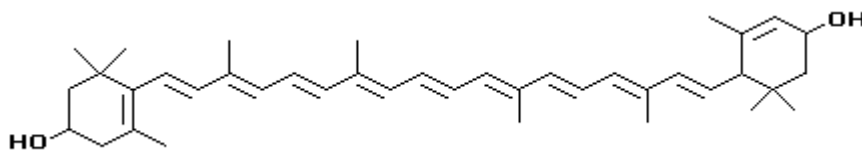


Figure 2.1: Lutein structure (Surai, 2003)

There is a basic structure of hydrocarbons running the length of the molecule and these are joined by conjugated double-bonds. At one or both ends of the carotenoids there are ring structures that can be substituted to give the molecule a different function. There are two groups of carotenoids; 'carotenes' are unsubstituted and therefore contain only carbon and hydrogen, and 'xanthophylls' are those that are substituted and contain oxygen. As a result of these structural differences, carotenes are non-polar compared to xanthophylls, and are lipid-soluble. Xanthophylls themselves are divided into 'keto-carotenoids', where the substitution is a ketone, and 'hydroxy-carotenoids',

where the substitution is a hydroxyl group (McGraw, 2006 in Hill, 2006 ch.5). The chemical formula for carotenoids is  $C_{40}H_{56}O_n$  where  $n$  is 0 in carotenes and  $n$  can be 1-6 in xanthophylls (Surai, 2003).

Birds are unable to synthesise carotenoids within their bodies as they lack the necessary precursor molecules (Hill, 2006 ch.12; McGraw and Hill, 2000). As a result birds must obtain carotenoids from their diet and then metabolise them into a form that they can utilise in their plumage. This immediately imposes an environmental pressure in the form of availability of carotenoids and is another way in which carotenoid display can be an honest signalling device. Some birds may have better foraging abilities than others and will ingest more dietary carotenoids and thus are able to exhibit more impressive plumage colouration. For females choosing a mate, plumage colouration can therefore be an honest indicator of foraging ability and the male's ability to find food for its offspring (Hill, 1991).

Carotenoids are difficult to absorb and the process requires large amounts of energy. In some cases infectious diseases, such as coccidia, can inhibit the absorption of carotenoids and other foods (Horak et al., 2004). Coccidia is a parasite that causes cysts to develop in the gut lining which make it thicker, a condition known as hyperplasia. This can stop the production of high density lipoproteins which transport carotenoids around the body (McGraw and Hill, 2000). Damage to the gut wall therefore prevents absorption of carotenoids. As carotenoids have to be transported across the small intestine with lipids, they must be eaten in a fat-rich diet. The multiple constraints on absorption of carotenoids are summarised in Figure 2.2.

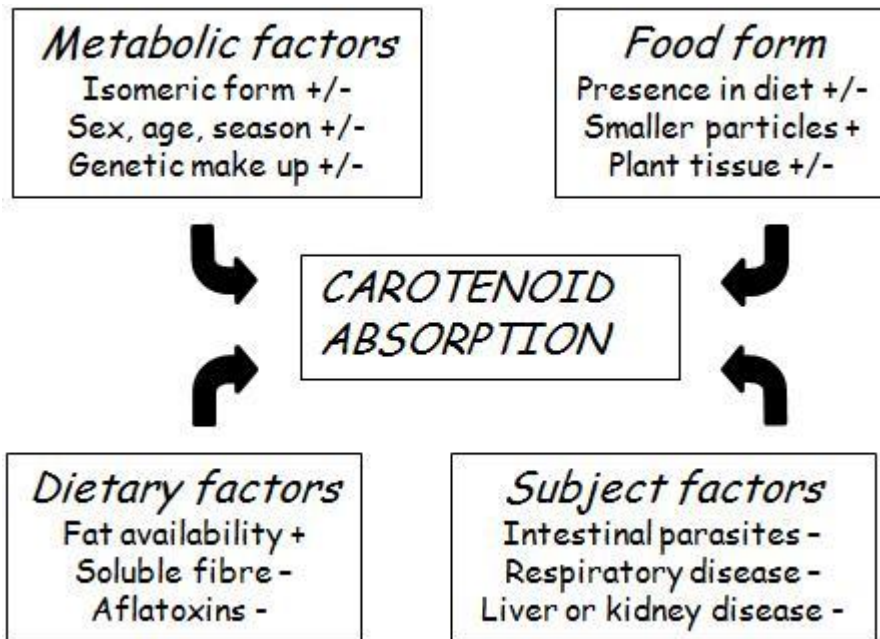


Figure 2.2: Diagram showing factors that affect carotenoid absorption, including metabolic factors, dietary factors, subject factors and food form as reviewed in Surai (2003)

### 2.2.2 Functions of carotenoids within the body

Carotenoids have important roles within the body that include eliminating peroxides and enhancing the immune system (Constanti and Møller, 2008). They do this by increasing the production of lymphocytes, enhancing the phagocytic properties of macrophages and neutrophils, as well as having a role in cancer immunity (Constanti and Møller, 2008). Carotenoids can also act as cell membrane stabilisers as their presence in the phospholipid bilayer of the small intestine means that they can trap free radicals and help maintain the integrity of the membrane (Surai, 2003). This is part of their role as antioxidants.

Antioxidants are molecules that are able to bond with free oxygen molecules that are circulating around the body. These oxygen molecules are known as free radicals and can be destructive to the tissues and organs. Free radicals are produced during metabolism and when mounting an immune response, being emitted when lymphocytes act to fight off an infection in the body. Carotenoids themselves can act as antioxidants as they are able to take up these free radicals and therefore reduce the damage that these cause during an immune response (Surai, 2003; McGraw et al., 2005; Biard et al., 2005).

One particularly important life history stage in birds relates to the large amount of oxidative stress produced during embryonic development (Surai, 2003). Studies have shown that female birds invest large numbers of antioxidants, particularly carotenoids, into egg yolk in order to aid the development of chicks. They do this by maintaining redox homeostasis during embryonic development and during the first few days after hatching (Blount et al., 2000; Surai, 2003). It is the carotenoid pigmentation in yolk that gives it its orangey appearance. McGraw et al. (2005) found that female zebra finches (*Taeniopygia guttata*) who invested more carotenoids into the yolk of their eggs, had chicks with better nesting survival, better fledging success and brighter ornamental colouration as adults. Similarly, Biard et al. (2005) found that nestlings from eggs laid by carotenoid supplemented females grew longer tarsi, had faster development of the immune system and grew brighter yellow feathers than controls. Carotenoids are therefore part of the “integrated antioxidant immune system” (Ewen et al., 2006b) and their interactions with other antioxidants are slowly being unravelled. Research has shown that dietary carotenoids when combined with other antioxidants, such as vitamins E and C, can enhance the protection of human cells against ultraviolet light. Combinations of antioxidants including carotenoids have also been shown to protect against and reduce lipid peroxidation (reviewed in Surai, 2003 ch.3). Specifically  $\alpha$ -tocopherol (a type of Vitamin E) and carotenoids work synergistically by reinforcing each other’s biological roles.

Some recent research has questioned the antioxidant properties of carotenoids (Constanti et al., 2007; Hartley and Kennedy, 2004). Hartley and Kennedy (2004) postulated that carotenoid-based display was in fact a signal of the functional properties of non-coloured resources. In order for a molecule to be classed as an antioxidant it has to be able to be oxidised without any detrimental effects, so that there is an overall net benefit in removing cause of oxidative stress. Carotenoids are long molecules that can be easily fragmented and when this happens, toxic compounds and more free radicals may be produced (Hartley and Kennedy, 2004). For example,  $\beta$ -carotene can be oxidised to produce toxic aldehydes that can have a negative effect on cell viability and replication. However, carotenoid-based display may in fact be indirectly advertising other antioxidant molecules that may have stronger antioxidant function but tend to be colourless. For example, Bertrand et al. (2006) looked at carotenoid-based bill colour in zebra finches and found that that birds who had brighter carotenoid colouration in their beaks, had higher levels of the non-pigmentary antioxidant, melatonin. Therefore it may be the case the brighter the carotenoid display, the better the quality of non-pigmentary antioxidants (Hartley and Kennedy, 2004) and therefore they are honest signals.

### 2.2.3 Metabolism of carotenoids for pigments

Metabolism is assumed to be a costly process and therefore adds to the value of these ornaments being used for honest advertising. The colour produced by carotenoids is dependent on the conjugated double-bonds, where the more bonds there are, the shorter the wavelength of light that can be absorbed (McGraw, 2006 in Hill, 2006 ch.5). Therefore, a molecule with many conjugated double-bonds will exhibit a red hue. In order to achieve these different colours dietary carotenoids may need to be metabolised. The metabolic process simply makes small structural changes to the carotenoid molecule. Greenfinches colour their feathers yellow using lutein and canary xanthophylls (McGraw, 2006 in Hill, 2006 ch.5). Lutein can sometimes be utilised without being metabolised, whereas the canary xanthophylls have to be synthesised from lutein by dehydrogenation:

LUTEIN → dehydrogenation → canary xanthophyll A → dehydrogenation → canary xanthophyll B

The enzymes that catalyse these reactions are as yet unknown, although it is assumed that some sort of dehydrogenase or mixed-function oxidase is responsible (Surai, 2003). Dehydrogenation occurs when the hydroxyl group of a hydroxy-carotenoid is converted into a carbonyl group. Other types of carotenoid are metabolised through oxygenation of the ring groups at the ends of the hydrocarbon skeleton.

### 2.2.4 Deposition of carotenoids for colour

If carotenoids do not need to be metabolised and can be deposited as pigment colouration in their current form, then they are transported to the relevant part of the body where they are deposited in the integument (McGraw, 2006 in Hill 2006 ch.5). McGraw (2004) found that in American Goldfinches, *Carduelis tristis*, the maturing feather follicle itself was the sight of metabolism of carotenoid pigments. As mentioned above, carotenoids can produce reds, yellows, oranges and pinks. Red integumentary colouration is by 4-oxo-carotenoids such as  $\alpha$ -doradexanthin, astaxanthin and canthaxanthin. Yellows are produced by xanthophylls such as canary xanthophylls, lutein and zeaxanthin (McGraw, 2006 in Hill, 2006 ch.5). Oranges and pinks are created by lower concentrations of the red and yellow carotenoids or combinations of them. It is unusual for a single carotenoid to make a colour; they more often co-occur with other carotenoids and pigment types (McGraw, 2006, in Hill 2006 ch.5).

### **2.2.5 Honest signalling and trade off with carotenoid pigments**

Carotenoids are ideal pigments in ornamental plumage and honest signalling as a good standard of health is needed in order to access them. They are hard to find, have to be eaten and they are difficult to absorb, therefore requiring a level of fitness to utilise them. Once deposited within the feathers, there is no way of retrieving carotenoids and there are fewer carotenoids left for other physiological functions. If a bird is fit and healthy, it is able to forage for carotenoid-rich foods and therefore have carotenoids available for plumage colouration. The bird must also have a good level of genetic resistance to diseases in order to choose carotenoids for pigment over their antioxidant and immune system roles. A trade off therefore exists whereby the bird must weigh up the relative importance of striking plumage and disease resistance and make a decision as to where to invest the carotenoids that are available. This costliness means that the signal is only displayed in the healthiest individuals and is therefore honest.

A male bird is therefore giving information through its plumage, about its health status to a female. This is a signalling device females can use to assess which birds are likely to have good heritable fitness for fighting infections, that can be passed on to their offspring. Females may use plumage colour as an indication of current disease status so that they do not catch a disease from a prospective mate. However it is better to base this judgement on physical disease symptoms as plumage colouration can only indicate past health status, at the time of the last moult. If a bird is looking healthy physically and has bright plumage then the female can assume that the male currently does not have a disease and is able to fight off future infections.

### **2.2.6 Key studies in bird plumage**

Following on from Darwin's theory on sexual selection, Hamilton and Zuk (1982) proposed that the quality of sexual display was a signal of parasite resistance. Several studies have been carried out that test whether carotenoid pigmentation predicts resistance to disease (reviewed in Møller et al., 1999). Some studies have also looked at whether varying the amount of carotenoids in the diet can have an effect on colouration of sexual characteristics. Olson and Owen (2005) found that dietary carotenoid availability correlated positively with plumage colouration but not with bare part colouration. They explained this by suggesting that life history and ecological factors were also playing a role and that plumage colouration was for dietary-mediated signalling, whereas bare part

colouration was a signal for some other, perhaps genetically influenced trait. Interestingly, they also found that this positive correlation was stronger for red plumage than yellow plumage.

Research into parasite-mediated sexual selection has resulted in both support and rejection of Hamilton and Zuk's (1982) hypothesis. A number of studies have been carried out on the house finch, *Carpodacus mexicanus*, as the expression of their ornamental traits can vary greatly (Hill and Montgomerie, 1994). Hill and Farmer (2004) challenged the immune system of house finches by inoculating birds with *Mycoplasma gallicepticum*, a novel pathogen. They found that males with redder plumage were able to clear the disease faster than those who were less red. Similarly, Nolen et al. (1998) found that male house finches with redder plumage were more likely to survive an outbreak of mycoplasmal conjunctivitis.

A study by Thompson et al. (1997) was the first study to use mark and recapture techniques to look at the effects of parasites on plumage colour over a length of time. House finches that had avian pox and feather mites present during the moult period were shown to be in poor physical condition and produced plumage that was less bright. Birds with low parasite loads, due to superior parasite resistance were in better condition and were able to grow brighter red plumage (Thompson et al., 1997). These studies then suggest that there is a positive correlation between carotenoid colouration and disease resistance. Horak et al. (2001) investigated haemoparasites in great tits (*Parus major*) and whether infection status was represented through plumage colouration. Uninfected yearlings were found to have brighter hues of their ventral, yellow pigmentation than infected yearlings, which therefore supports Hamilton and Zuk's (1982) hypothesis. However, in older males infected birds had higher hue values than uninfected birds. If a bird had come into contact with the disease at an earlier age, it may be that they survived and now have the disease in a chronic form. They may require just a small immune response to keep the disease at bay, showing little or no signs of infection. An earlier disease outbreak may also have filtered out some birds with less resistant immune systems and others with weak immune systems may have not come into contact with the disease at any stage throughout their lives (Horak et al., 2001).

### **2.3.1 Infectious diseases**

As with all animals, birds are susceptible to a number of infectious diseases. Infectious diseases are acquired following contact with an external pathogen present within an infected individual or the

environment (Ball, 1982). They can be transmitted through the media of air or water. Birds are generally fairly social and use communal feeding stations and water sources; therefore it is easy for diseases to be transmitted from one to the other. The main source of diseases in birds is from other birds rather than from other animals such as rats and squirrels which are known for their ability to harbour and spread disease.

Infectious diseases can be pathogenic and can be either chronic or acute. They are detrimental to the health of the bird and may cause death. Disease lowers an animal's fitness and it may be more prone to such things as predation and hunger. Infectious disease is therefore an important parameter affecting a bird's survival.

### **2.3.2 Emergent infectious diseases**

Emergent infectious diseases are those that are increasing in prevalence and are becoming more widespread and common. The birds that are catching them have not been exposed to the particular pathogen before and therefore it is harder for their immune system to fight off infection. Emerging infectious diseases can be particularly aggressive because of this and have a high virulence. For example, Ewen et al. (2007) looked at the effect of *Salmonella typhimurium* on a reintroduced population of the hihi or stitchbird (*Notiomystis cincta*) on Tiritiri Matangi island, New Zealand. This was an emerging infectious disease and a novel pathogen for these birds, as it had not previously been found on the island. It was first noticed in 2006 with the finding of eight freshly dead hihi that showed typical signs of salmonella in autopsy. The disease faded out as the susceptible individuals were removed (estimated about 30% of adult population died), but it still may be carried in resistant individuals on the island (Ewen et al., 2007). Management and monitoring of the population must continue so that this problem cannot arise again in the future. This example highlights the problems that can arise when such a virulent novel pathogen enters population, particularly in an endangered species.

Both infectious diseases and emerging infectious diseases are currently causing problems in UK passerines. These are salmonella and trichomonas respectively. Salmonella is transmitted through faecal contamination of food (Van Oort and Dawson, 2005) and trichomonas is transmitted through saliva contamination food. People often have bird feeders in their gardens and these are prone to a build up of contaminants. Bird feeders that are not washed risk a build up of disease contaminated

faeces that can be picked up by any bird that comes to feed at the bird table (GBHi, Best Practice Guidelines).

### **2.3.3 Salmonella**

Salmonella in wild birds is typically caused by the bacteria *Salmonella typhimurium*. *S typhimurium* infections cause swelling and lesions in the crop lining that generates a lumpy appearance. The spleen may be swollen, there may be necrosis of the digestive organs (Van Oort and Dawson, 2005) and physically the bird's feathers look ruffled and in poor condition. There is severe weight loss and at the time of death the birds are typically emaciated. This is partly due to the disease's detrimental effect on the gut, causing the bird to defecate frequently. The faeces contain the salmonella bacteria and therefore frequent defecation is the disease's way of spreading itself to other birds (GBHi, salmonella advice sheet). Prior to death, birds are lethargic and tend to stay near feeders, so they do not have to forage for food. This increases the risk of contamination for other birds visiting the feeding station. Salmonella can easily be grown in a lab and therefore can easily be diagnosed.

### **2.3.4 Trichomonas**

Trichomonas is caused by a protozoan parasite called *Trichomonas gallinae* and is found to affect bird populations throughout the world. It affects the upper digestive tract of birds by causing lesions and build up of necrotic tissue (Becki Lawson, pers. comm.). It tends to be more common among young birds and is well known as a disease of pigeons and doves. Pigeons pass it to their young by feeding them crop milk, which is where the parasite lives. In adult birds it is transmitted through saliva, and raptors can catch it from eating infected birds. As well as the visible lesions in the throat, symptoms include wet breast feathers, weight loss and badly maintained plumage due to lack of grooming. The crop lining is thin and the oesophagus and crop can get occluded. The observed disease outbreaks tend to peak at the end of summer or beginning of autumn. As with salmonella, weight loss is a symptom, due to lack of foraging ability. Sick birds often have large amounts of food debris in their throats that they are unable to swallow and digest due to the lesions in their throat.

## 2.4 Garden bird project

There has been an increase in the number of people interested in providing food for garden birds, with a greater concern for the welfare of their local wildlife. However, food has to be provided hygienically otherwise the benefits of feeding the birds are countered by the increased opportunity for disease transmission. The cleaning of feeding stations has not been done in the past and this has led to concerns being raised about the increased risk of infectious diseases to garden birds. As a result of this concern, the Garden Bird Health Initiative was set up in 2003 by the Universities Federation for Animal Welfare to provide guidelines to the general public on best feeding practices and to promote research in the area of emerging infectious diseases ([www.ufaw.org.uk/gbhi.php](http://www.ufaw.org.uk/gbhi.php)). A monitoring network has been running since April 2003 and members of the public and volunteers have sent in 1,566 birds to the GBHi to have post-mortem examinations carried out on them. Of these birds, 539 have been greenfinches which constitute a considerable proportion of all dead birds found. Greenfinches are therefore highly susceptible to infectious diseases and are to be the study species of this project.

### 2.5.1 Greenfinches

Greenfinches, *Carduelis chloris*, are seed eating birds belonging to the finch family, Fringillidae (Lindström and Lundström, 2000). They are Passeriformes, a very diverse order also known as 'songbirds' or 'perching birds'. A healthy adult greenfinch should weigh about 30g (Grant et al., 2007) and measures about 15cm in length (Birdlife International, 2004). They are gregarious, meaning that they tend to be sociable and live close to others of the same species. Greenfinches have a wide distribution, being found throughout Europe, as well as North Africa and western parts of Asia, and they have also been introduced into Australia, New Zealand and Uruguay (Birdlife International, 2004). The main body colour of a greenfinch is an olive green, with males having bright yellow breast and belly feathers and females having a duller yellow on their breast feathers and often lacking any yellow plumage on their belly (Aguilera and Amat, 2007). Males also have bright yellow plumage on their primary feathers, primary coverts and the sides of their tail feathers (Horak et al., 2004) which females have but it tends to be duller.

### 2.5.2 Greenfinches, infectious disease and sexual selection

One focus of research on greenfinches and parasite mediated sexual selection relates to the Sindbis virus (Lindström and Lundström, 2000; Lindström et al., 2001; Lindström, 2004). Lindström and Lönstrom (2000) injected Sindbis virus into male greenfinches and carried out blood tests subsequently to ascertain the clearance rate of the virus and the level of virus in the body during infection. They found that males who had larger and brighter patches of carotenoid-based yellow plumage on their tails were able to clear the virus faster and had lower virus titres at peak infection. The ability to clear the virus was a measure of immune functioning and this varied greatly between greenfinches. Lindström and Lundström correctly pointed out that it is unknown whether this ability to control the Sindbis virus is heritable and can therefore be related to sexual selection.

In addition, several studies have injected one group of birds with sheep red blood cells to induce a humoral immune response and injected another group with PHA (plant-lectin phytohaemagglutinin) to induce a cell-mediated immune response (Saks et al., 2003; Saino et al., 2003a; Saino et al., 2000; McGraw and Ardia, 2003). Saks et al. (2003) plucked 2 to 4 breast feathers from male greenfinches and found that brighter yellow plumage in these feathers was associated with a stronger humoral immune response. They did not however find any significant relationship between PHA induced cell-mediated immune response and feather brightness. The methods that are used when measuring immune function have been questioned by Saks et al. (2006) and they recommend a cautious interpretation of results when using these methods to look at parasite resistance.

Horak et al. (2004) and Horak et al. (2006) have both looked at the effect of coccidiosis on the appearance of greenfinches. In both studies birds were experimentally infected with the coccidia species, *Isospora lacazei*. Horak et al. (2006) found that birds who had naturally low infection status retained this even after being experimentally infected with various different strains. This meant that these birds were much better at resisting pathogens. Horak et al. (2004) found that infected greenfinches had reduced expression of plumage colouration and concluded that this was because of a deficiency of carotenoids available for deposition in feathers.

Merila et al. (1999) and Harper (1999) both found that parasite load was negatively correlated with plumage colour in greenfinches when investigating the effects of haemoparasites and feather mites respectively. Similarly, Aguilera and Amat (2007) determined that activating the immune system of

greenfinches reduced the number of carotenoids circulating in the blood and also that chroma of breast plumage was negatively correlated with immune response.

Ornamental plumage colour may reveal an individual's susceptibility to both infectious and emerging infectious diseases, as predicted by honest signalling. A study was carried out on common redpolls to test this hypothesis (Van Oort and Dawson, 2005). They used plucked feathers from birds that had died of salmonella and compared the carotenoid pigmentation to that of live, healthy birds. Their results actually found that adult male redpolls that had more developed ornamental plumage were more likely to have died from salmonella. This does not support the theory of honest signalling, but Van Oort and Dawson (2005) proposed that the carotenoid ornamentation of male redpolls was actually a sign of dominance. The brighter birds had dominated highly contested feeding stations and were therefore putting themselves at a greater risk of exposure to salmonella. In this study, I will test these ideas by quantifying the plumage colouration on a sexually selected trait that is well studied in the greenfinch.

## **2.6 How to measure colour**

In order to make sense of the colours that are displayed by birds, we use several measurements. The colour we perceive, such as red or blue, is known as 'hue' (Montgomerie, 2006 in Hill, 2006 ch.3). It is also referred to as the spectral location, as it is usually measured as a position or wavelength along the visible spectrum. It may also be measured as an angle in degrees, around a colour wheel. The 'purity' of the colour is known as the 'saturation' or 'chroma' (Montgomerie, 2006 in Hill, 2006 ch.3). When the colour is fully saturated and completely pure, it is only made up of one wavelength of light. The lightness of the colour is referred to as 'brightness' and is measured by the intensity of the radiance, or amount of reflection, coming from a surface (Montgomerie, 2006 in Hill, 2006 ch.3). Figure 2.3 shows each of these in the form of a Munsell Colour System. This does, however, only show colours that are within the human visible range whereas birds are able to see into the UV and therefore they also use UV colours in their plumage.

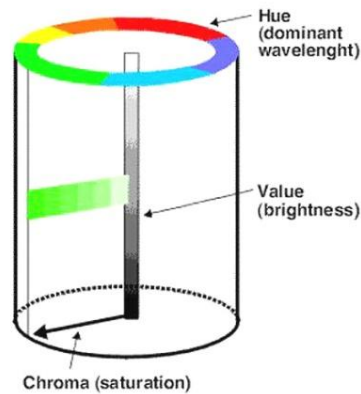


Figure 2.3: Munsell Colour System showing hue, brightness and chroma ([webvision.med.utah.edu/KallColor.html](http://webvision.med.utah.edu/KallColor.html))

Colour can be measured in numerous ways, such as comparison with colour cards or from photographs, but these are rather subjective although widely used. A more accurate (and less subjective) approach is to use a spectrometer. Spectrometers also have the benefit of being able to record spectral reflectance within the UV wavelengths. Previous research has shown that carotenoid signalling in feathers is best explained by chroma, hue and sometimes brightness in the yellow wavelengths but yellow colouration also often has peaks in the UV wavelengths. Measures of yellow chroma, yellow brightness, UV chroma and UV brightness were therefore calculated in this study, following the methods and justification in (Thorogood et al., 2008).

We can see then that carotenoid-based greenfinch plumage can function as an indicator of quality and that males may trade ornamental colouration against immune response (Aguilera and Amat, 2007). However, studies are still providing conflicting evidence and research to date on greenfinches has been on captive birds. Greenfinches are a good study candidate as they are sexually dichromatic and have sexual ornamentation that is carotenoid-based. This thesis is going to examine wild greenfinches that have been parasitized to the extent that it has caused their death and compare their carotenoid-based plumage colour with greenfinches that have died from causes other than infectious disease.

### **3. Method**

#### **3.1 Source of Samples**

Dead greenfinches were collected as part of the Garden Bird Health Initiative (GBHi) from 2005 to 2008 (Becki Lawson, unpublished data). This project was developed to record and investigate the causes of garden bird mortality and disease across Great Britain. A systematic monitoring network of participants from the BTO/CJ Garden BirdWatch scheme was set up to report sightings of garden bird morbidity or mortality on a weekly basis. Participants contacted a nominated, regional diagnostic laboratory in order to arrange for birds to be submitted for post-mortem analysis ([www.zoo.cam.ac.uk/ioz/projects/garden\\_bird\\_health\\_initiative](http://www.zoo.cam.ac.uk/ioz/projects/garden_bird_health_initiative); also see Figure 1). From April 2005 to March 2008, the GBHi carried out post-mortem examinations on 1566 birds, of which 539 were greenfinches. Dead birds were submitted largely to the Institute of Zoology's pathology department located at the Zoological Society of London. Accompanying the dead birds were data on the location where they were found and the date. Figure 1 shows the distribution of greenfinches throughout the UK that were used in this project.

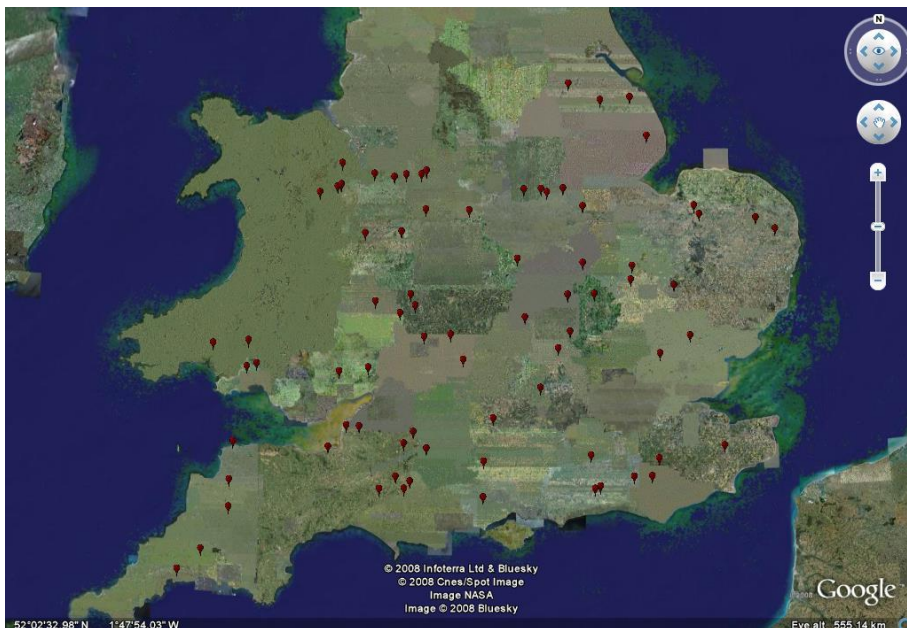


Figure 3.1: Map of the UK showing distribution of greenfinches used in this project. All greenfinches were found throughout England and Wales.

### 3.2 Post-mortem examination

Birds were sexed and aged initially from their plumage, with sex being confirmed by locating the gonads where possible. Age was divided into three categories juvenile, first year and adult. Juvenile was considered to be from when primary feathers are fully grown until post-juvenile moult is complete. First year and adult were distinguished by plumage and skull ossification where possible, with adult birds having more advanced skull ossification. Birds were weighed and wing length and maximum tarsal length were also recorded. The bird's eyes and throat were checked for blood and lesions, signs of trichomonas. The breast feathers, skin and flight muscles were then removed and the crop and intestine were examined for signs of trichomonas and salmonella. Swabs were taken from the throat and intestine and bacterial cultures were grown and identified to see whether an infectious disease was present and if so, what type. A screen was also carried out to see if an intestinal parasite was present. Pathogens were identified as trichomonas, salmonella, intestinal parasites or some other undetermined disease. Birds were also presented that had died from various types of trauma not associated with disease (e.g. predation, hitting a window). These samples represent individuals that were otherwise healthy and I consider them as control samples against those dying of disease. Results from post-mortem examinations along with the date and location of where the birds was found, were then collated onto a database. I obtained access to this data from the Garden Bird Project with kind permission from Becki Lawson.

### 3.3 Colour analysis of feathers

In addition to the standard post-mortem analyses, the outer most feather on either side of the tail was removed for later colour analysis. These feathers were chosen as they are the most exposed part of the tail and therefore are likely to be representative of the colour signal of the whole tail (Saks et al., 2003). Feathers were sealed in separate plastic bags for each bird and stored in a freezer from the time of collection until use. By storing them in the freezer, the feathers were kept in the dark away from sunlight thereby reducing the chance of the colour fading (Saks et al., 2003; Horak et al., 2004).

The right outermost tail feather was also removed from the tails of live, healthy greenfinches in New Zealand during the late Southern Hemisphere summer of 2004. The birds were caught in mist nets on agricultural land in the main North Island and the habitat was very similar to the agricultural landscape of the UK (grass pastureland for sheep and cattle grazing and orchards). Feathers from New Zealand greenfinches were stored in separate plastic bags in the dark at room temperature from the time of collection until measurements were carried out.

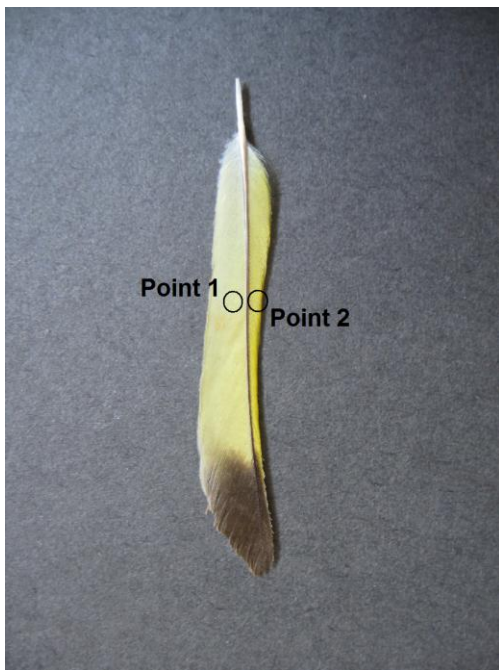


Figure 3.2: Outer right tail feather from adult male greenfinch mounted on black card for colour analysis. The circles show the standard locations where colour measures were taken across feathers from different individuals.

The colour measurements were carried out on feathers mounted on black card in the post-mortem laboratory of ZSL, to ensure constant lighting, using an Ocean Optics USB2000 Miniature Fiber Optic Spectrometer with illumination by a DT mini-lamp. My protocol followed that of Saks et al. (2003), whereby measurements were taken either side of the central feather barb and on both the right and left tail feathers (Figure 2). Three repeated measures were taken at each position on each feather using a 200 $\mu$ m quartz fibre optic cable. The spectrometer was calibrated using a standard WS-2 white reference tile and the black piece of card that the feathers were mounted on. A light-proof cap was fitted to the end of the probe to ensure the light hit the feather at a constant angle of 90°. The spectral reflectance range was 300 to 700nm with measurements at each 1nm interval. Spectral

reflectance data was passed into a computer that converted the spectral values into values of yellow chroma (YC), yellow brightness (YB), UV chroma (UC) and UV brightness (UB). Yellow chroma is the proportion of total reflectance that has a yellow hue across the whole spectrum and was therefore calculated by dividing reflectance in the yellow wavelengths by the total reflectance ( $R_{550-625}/R_{300-700}$ ). Yellow brightness was then calculated as average reflectance in the wavelengths 550-625nm and UV brightness was calculated as the average reflectance in the wavelengths of 300-400nm. UV chroma was calculated as the proportion of total reflectance in the wavelength of 300-400nm.

### 3.4 Data analysis

The repeatability of the measurements taken was assessed to determine if there was more variation between individuals than there was between repeated measures on the same individual. This was done using ANOVAs and following protocol presented by Lessells and Boag (1987), giving repeatability values between 0 and 1 where 0=no repeatability and 1=complete repeatability. All statistical analyses were carried out using the software program, R (Crawley, 2007) The values from the same points on the left and right feathers of the same individual were also compared to see if there was any difference in colouration across the tail. If the repeatability of these measurements was found to be high, I assume the averaged colour parameter values for each point per bird is an accurate representation of their colour profile.

The different colour variables that were recorded by the spectrometer were compared with one another to see if there were any strong linear correlations. Pearson's Product-Moment Correlation Coefficient was calculated for all Point 1 scores against each other and then colour scores at Points one and 2 were compared. If strong relationships were seen, then not all colour measurements needed to be included as separate variables in the main data analyses because they would be capturing the same variability in measured dependent variables.

Feather samples taken from healthy New Zealand greenfinches and from UK birds which had died of trauma were used as controls when comparing colour against diseased UK greenfinches. In order to provide an indication of whether colour differed between non-diseased birds from New Zealand and the UK (suggesting that these groups are otherwise comparable), a generalised linear model was

used to compare the yellow chroma scores at Points 1 and 2 (YC1 and YC2) of New Zealand feathers and UK trauma feathers.

In order to test whether the variables of age, sex and habitat could explain the dependent variables of disease status, trichomonas status, salmonella status and intestinal parasite status, four generalised linear models were run. Disease status was considered positive if the bird had a positive diagnosis of trichomonas, salmonella or intestinal parasites. Trichomonas status was considered positive if there was a confirmed diagnosis of trichomonas from the post-mortem analysis. Trichomonas status was negative if death was caused by salmonella or trauma, with no diagnosis of trichomonas. Similarly, salmonella status was considered positive if there was a confirmed diagnosis of salmonella from the post-mortem analysis. Salmonella status was negative if death was caused by trauma or trichomonas, with no diagnosis of salmonella. The intestinal parasite screen from the post-mortem analysis was able to indicate the presence of absence of intestinal parasites. The dependent variables were all made up of binary data and therefore the error structure was binomial. The explanatory variables were categorical, for example age could be "juvenile", "1<sup>st</sup> year" or "adult". Habitat was categorised as "Urban", "Suburban" or "Rural" which was determined using the location data for each bird found. The models also looked at whether there was an interaction between age and sex that could have an effect on the dependent variables. Stepwise deletion of terms from the model gave a minimal adequate model that contained the significant variables ( $p$  values  $<0.05$ ) when testing deviance changes against the chi squared distribution.

A further four generalised linear models were run with the colour variables YC1, YC2, yellow brightness at point 1 (YB1) and yellow brightness at point 2 (YB2) as explanatory variables, against the same dependent variables as the first four models. These models were run to test if colour was associated with disease status, salmonella status, trichomonas status or death from trauma. The colour variables were continuous data sets, but the error structure was binomial as the dependent variables were still binary. Again, stepwise deletion of terms gave the minimal adequate model showing the significant variables only. In this second set of models only adult male greenfinches were included as females and juveniles tend to lack the full yellow markings in their feathers (Saks et al., 2003) and my aims are to directly test the honest signalling hypothesis of male greenfinch plumage signals.

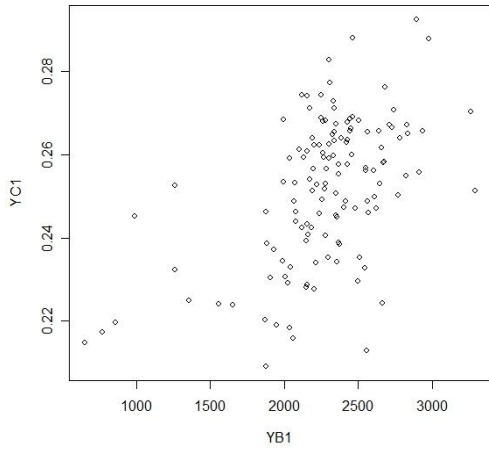
#### **4. Results**

The repeatability of the colour measurements made within a point on a given feather was very high (all > 0.9) showing I was very repeatable at recording colouration. Even when I combined colour measures from the same point on two representative feathers of an individual the repeatability scores were still high (all > 0.7,  $p < 0.05$ ). I therefore combined left and right feathers and produced an average of the six repeat measures to give a final colour score per bird at point 1 and point 2.

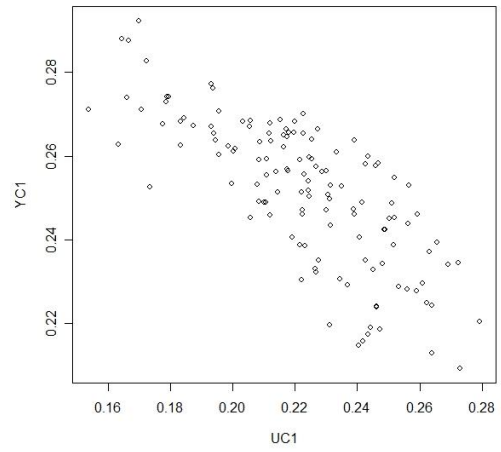
Colour variables showed various relationships between one another that meant some could be excluded from the final analyses because they were not independent (Table 4.1, Fig. 4.1). Notably YB1 and UB1 were strongly positively correlated with one another as were YB1 and YB2. YC1 was strongly negatively correlated with UC1.

<b>Colour variables</b>	<b>Correlation Coefficient</b>	<b>Degrees of freedom</b>	<b>p value</b>
YC1 : YB1	0.5186298	130	<0.05
YC1 : UC1	-0.7559269	130	<0.05
YC1 : UB1	-0.2588789	130	<0.05
YB1 : UC1	-0.1520575	130	<0.1
YB1 : UB1	0.6388484	130	<0.05
UB1 : UC1	0.6277067	130	<0.05
YC1 : YC2	0.5103031	109	<0.05
YB1 : YB2	0.5599633	130	<0.05
UC1 : UC2	0.6114896	130	<0.05
UB1 : UB2	0.4643901	130	<0.05

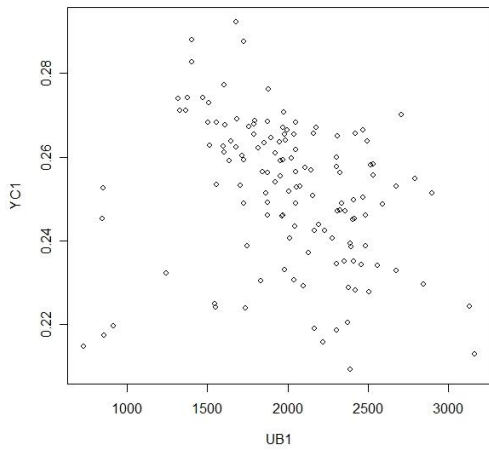
Table 4.1: Comparison of yellow chroma, yellow brightness, UV chroma and UV brightness at point 1 and point 2 on greenfinch feathers.



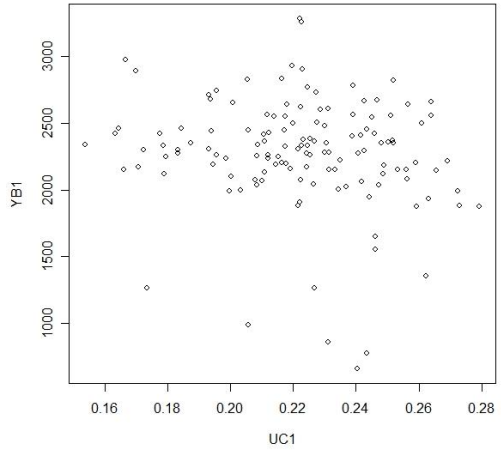
a)



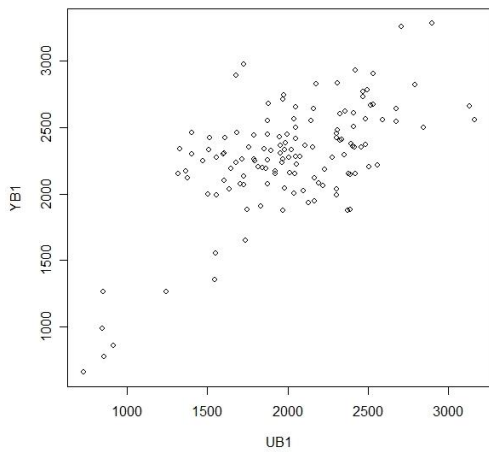
b)



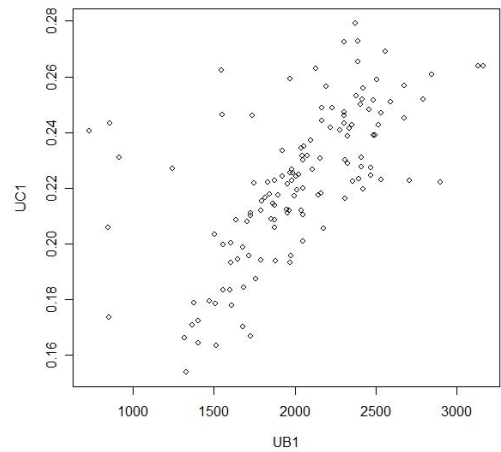
c)



d)



e)



f)

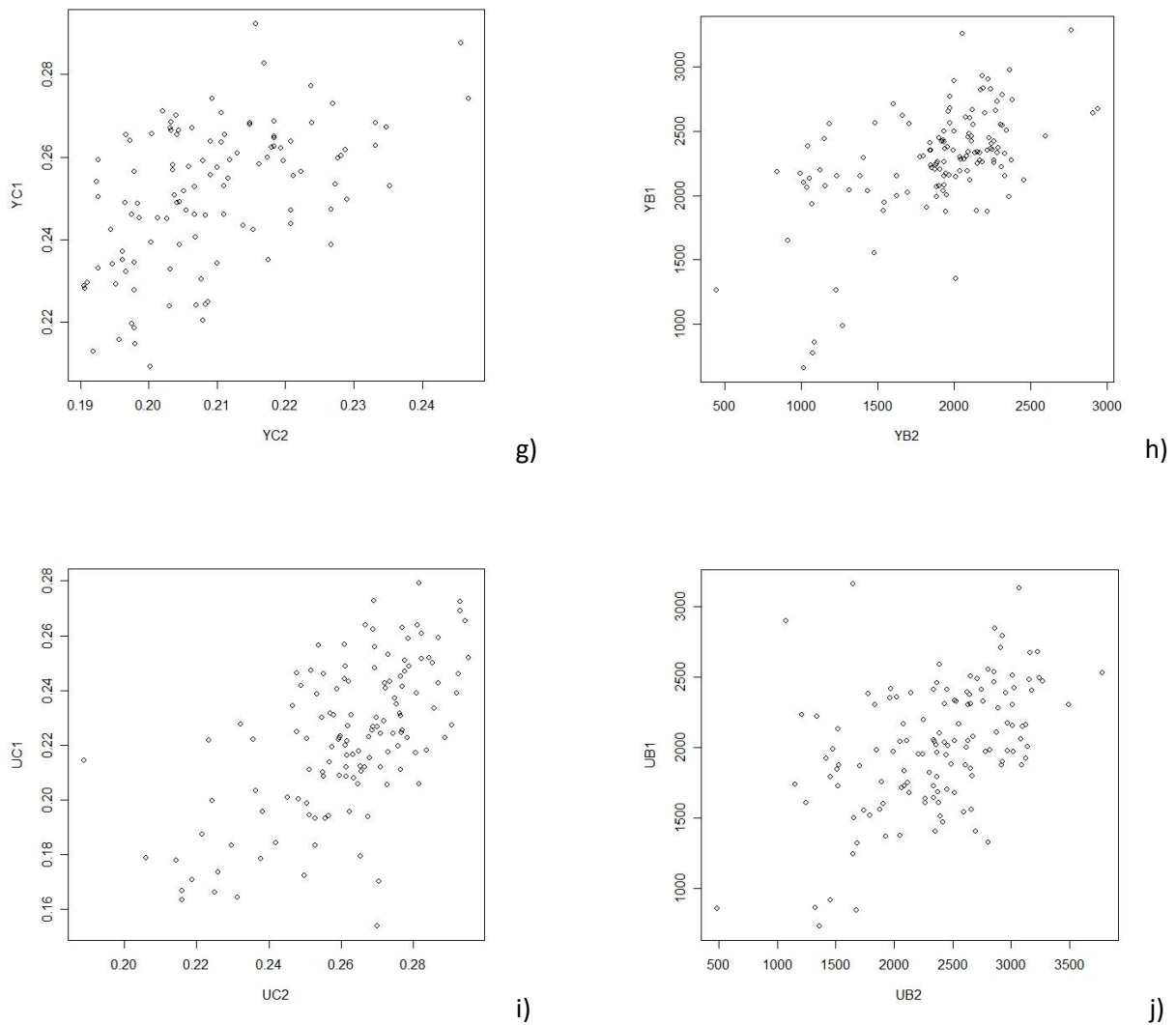


Figure 4.1: Scatter diagrams showing correlations between yellow chroma, yellow brightness, UV chroma and UV brightness. a) YC1:YB1, b) YC1:UC1, c) YC1:UB1, d) YB1:UC1, e) YB1:UB1, f) UB1:UC1, g) YC1:YC2, h) YB1:YB2, i) UC1:UC2 and j) UB1:UB2

All colour scores were positively correlated between points 1 and 2, and all colour scores showed strong correlation with the exception of YB1:UC1 (Figure 4.1(d)). For this reason I chose to use only yellow chroma and yellow brightness as these parameters captured most variation in both ultra violet chroma and ultraviolet brightness. However, the correlations between points 1 and 2 were a little weaker than the repeatability scores within Points 1 and 2 and therefore they were kept as independent variables.

New Zealand birds were found to have significantly higher values of yellow chroma than UK trauma birds ( $p < 0.05$ ,  $\chi^2 = 0.0000579$ , d.f. = 9) as is illustrated in the boxplots in Figure 4.2. As a result of this difference, the New Zealand birds could not be used as controls and were excluded from the main analyses.

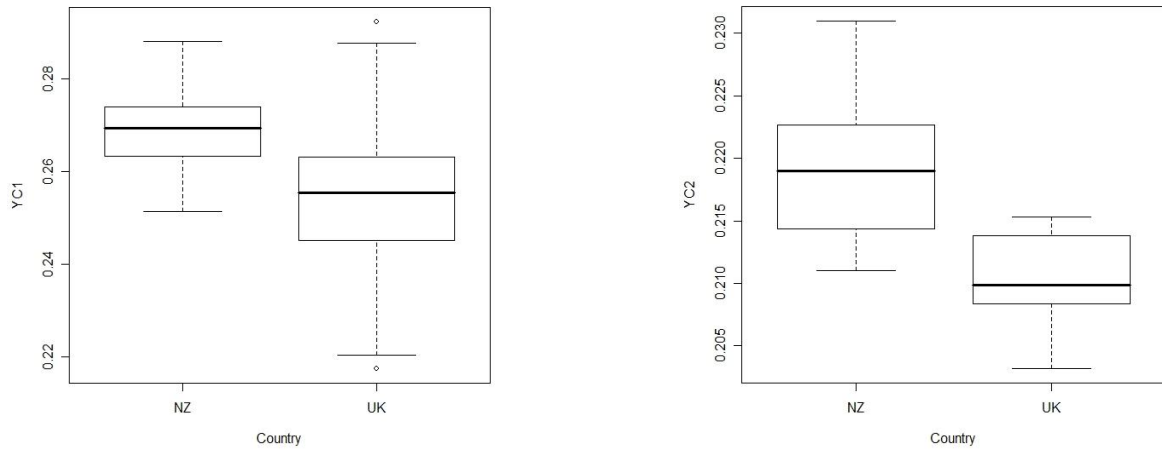


Figure 4.2: Comparison of yellow chroma for points 1 and 2 on feathers in New Zealand and UK trauma greenfinches

Dependent variable	Explanatory variable(s)	$\chi^2$	p
Disease status	None	-2.259	0.133
IPS	None	-1.861	0.173
Salmonella status	Age	-7.686	0.021
Trich status	Sex	-8.925	0.003
	Age	-12.134	0.002

Table 4.2: Results from four generalised linear models, with the parameters sex, age and habitat as explanatory variables and disease status, intestinal parasite status (IPS), salmonella status and trichomonas status as dependent variables. The table shows the values for chi squared and p from the minimal adequate models, with all having d.f. = 1. Where the variables were significant, they have been named as there is a lower p value, being <0.05. Where there were no significant variables, the p value is higher, being >0.005.

Adult greenfinches were more likely to have salmonella or trichomonas than juveniles or first year birds (Table 4.2; Figure 4.3a and Figure 4.3b). In the case of trichomonas, males were also significantly more likely to have the disease than females (Table 4.2; Figure 4.3c). Disease status and intestinal parasite status were not correlated with any of the variables included in the first four models including, sex, age and habitat (Table 4.2). Habitat also did not explain salmonella or trichomonas status, and age and sex did not interact to explain any of the dependent variables.

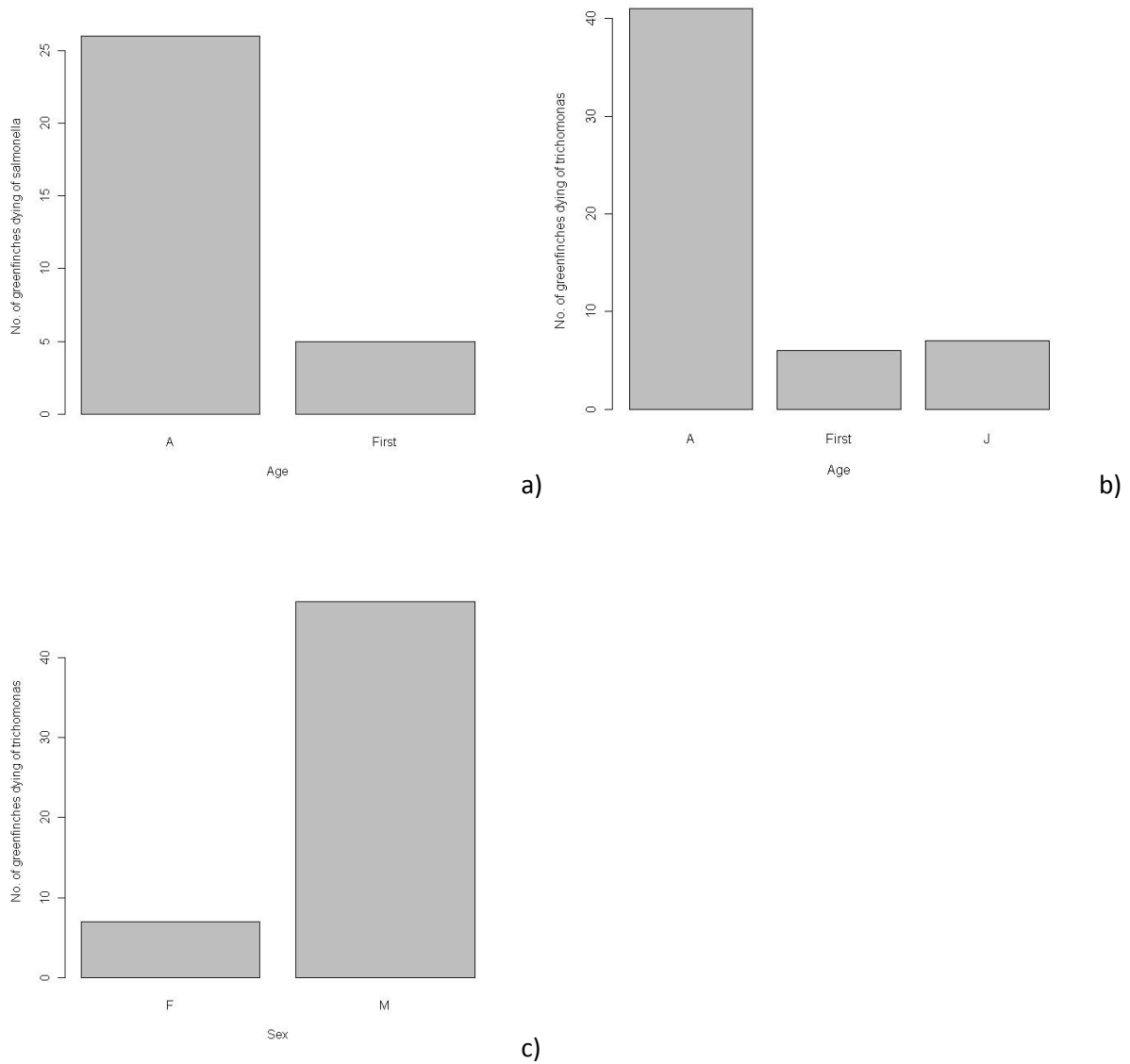


Figure 4.3: Proportional prevalence of diseases compared between age and sex groups. a) salmonella versus age; b) trichomonas versus age; c) trichomonas versus sex. The dark area represents females and the light area represents males.

Dependent variable	Explanatory variable(s)	$\chi^2$	p
Disease status	YC1	-4.075	0.044
IPS	None	-0.807	0.369
Salmonella status	YB1	-5.189	0.02
Trich status	YB1	-6.027	0.014

Table 4. 3: Results from four generalised linear models, with colour parameters YC1, YC2, YB1 and YB2 as explanatory variables and disease status, IPS, salmonella status and trichomonas status as dependent variables. The table shows the values for chi squared and p from the minimal adequate models, with all having d.f. = 1.

Among adult male greenfinches, the minimal model predicting disease status included chroma at point 1 (Table 4.3; Figure 4.4a) showing that greenfinches with a higher chroma value were more likely to have a disease. Brighter birds were less likely to have trichomonas, but more likely to have salmonella. YB2 and YC2 did not show any independent effects on any of the disease or parasite status variables, and intestinal parasite load was not significantly related to any colour measure.

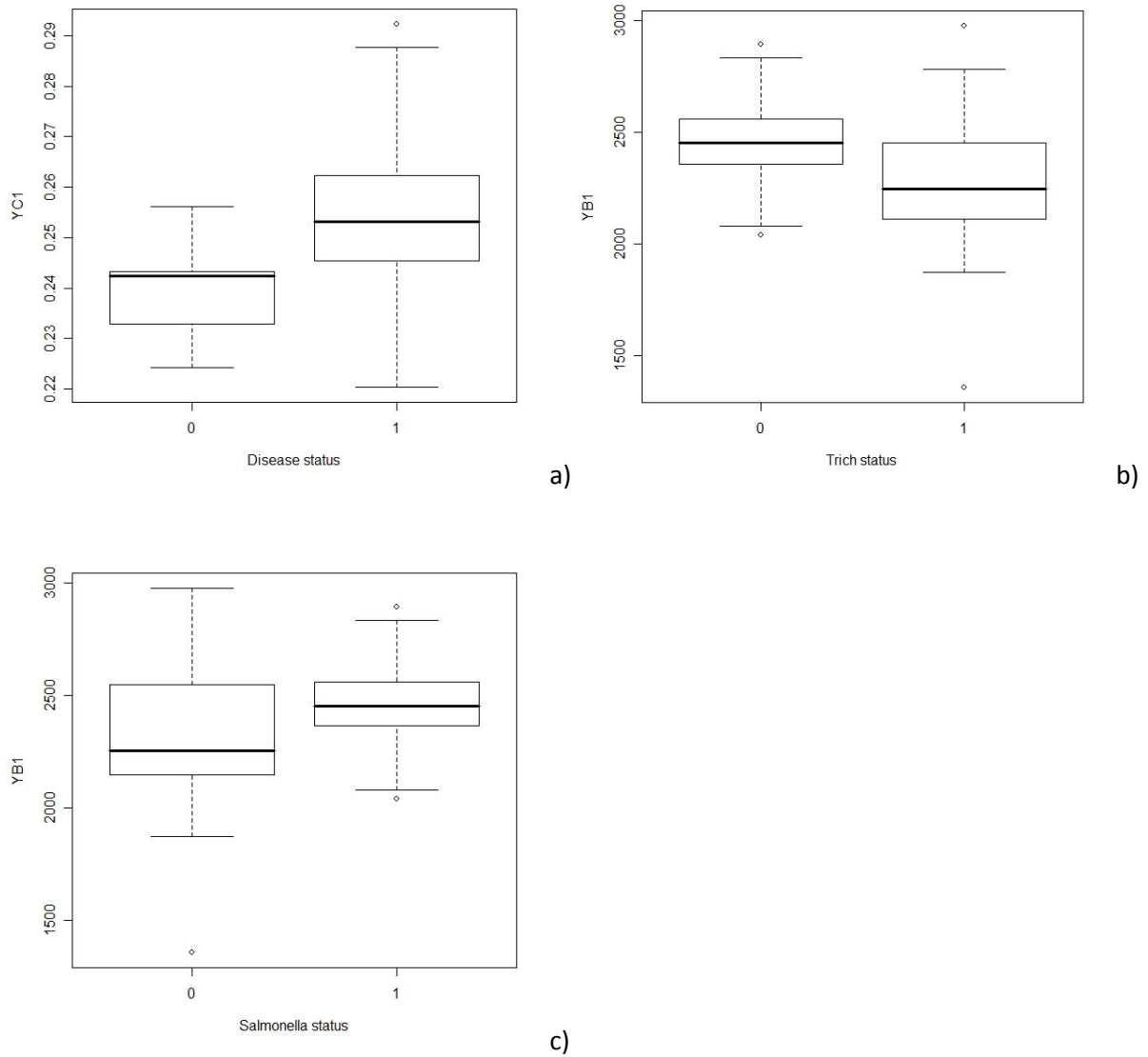


Figure 4.4: Boxplots showing colour measures against disease status. a) YC1 with respect to overall disease status; b) YB1 with respect to trichomonas status; c) YB1 with respect to salmonella status. Heavy bars represent the median colour measurements, the bottom and top of the boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles respectively, whiskers represent 1.5 times the interquartile range of the data, and points represent the outliers (Crawley, 2007).

## **5. Discussion**

In this study I examined whether carotenoid signalling in the greenfinch (*Carduelis chloris*) was associated with the probability of having an infectious disease. I also looked at whether other variables such as age, sex and habitat had an effect on the likelihood of having an infectious disease. The results showed that none of sex, age or habitat explained disease status, when disease was a single category that included trichomonas, salmonella and intestinal parasites. When the model was rerun with these as individual dependent variables, intestinal parasite status (IPS) was also not correlated with sex, age or habitat. Adult greenfinches, however, were more likely to have died from trichomonas or salmonella than juveniles or first year birds, and males were much more likely to have died from salmonella than females. I had predicted that birds in rural areas would be less likely to die from an infectious disease compared to birds in urban areas, but no association was found. Following on from this, I found that in adult male greenfinches, yellow chroma was positively associated with disease status. Similarly, birds with higher values of yellow brightness were more likely to have died from salmonella. However, brighter birds were less likely to have died from trichomonas, which supports my hypothesis that brighter carotenoid ornamentation in birds is an honest indicator of strong disease resistance. The findings for salmonella and disease status do not support my hypothesis and show that the most ornamented individuals were more likely to have died from an infectious disease.

Several other studies have looked at the relationship between disease status and sex, age and habitat. Boal et al. (1998) found that there was no difference between urban or rural environments on trichomonas status in adult Cooper's hawks, *Accipiter cooperii*. This supports my finding that habitat did not have any effect on trichomonas status, or any other dependent variable. Boal et al. (1998) did, however find that nestlings were more infected with trichomonas in urban areas than rural areas. This project did not include nestlings and therefore this pattern could not be tested. Grant et al. (2007) found that there was no relationship between salmonella status and sex or age in greenfinches. This is in contrast to my findings that show adult male greenfinches are more likely to have salmonella. Age was not related to immune function in the great tit (*Parus major*) in a study by Dufva and Allander (1995). Although not directly testing disease status, immune function is an indicator of how well a bird can resist a disease and therefore this study shows that immune function and disease resistance potential does not change with age. A study on Pekin ducks (*Anas platyrhynchos domesticus*) and Roman geese (*Anser anser domesticus*) showed that younger birds

had a higher prevalence of salmonella (Yu et al., 2008), which is the opposite of my findings. I suggest that adult birds are more susceptible as they are more exposed to pathogens. This is primarily because they may be dominant at feeding stations, where there is a higher likelihood of disease transmission. Other studies have linked disease susceptibility to dominance such as Van Oort and Dawson (2005).

With regards to sex differences, Richner et al. (1995) found that male great tits had a higher malarial parasite load than females, which supports my finding that males are more likely to have salmonella than females. The majority of studies that have looked at disease and its explanatory parameters, have only investigated adult male birds (Horak et al., 2004; Horak et al., 2006; Lindström and Lundström, 2000), with the exception of Aguilera and Amat (2007) who used first year males. This automatically excludes any possible interaction that sex and age may have on disease status. The differences I have found between adult males compared to females or younger birds could, of course, be due to a sampling bias. However, I consider this unlikely as dead birds were generally found near to or on feeding stations and therefore would be easily seen regardless of any possible colour or physical differences (Van Oort and Dawson, 2005). I suggest that more research needs to be done to understand how sex, age and habitat can influence the susceptibility of individuals to trichomonas, salmonella and intestinal parasites.

As would be expected, the amount of yellow on the feather, varied along its length. This was why point 1 and point 2 were kept separate. Feathers from the same bird did however show consistent colour measurements along the length of the feather. Yellow chroma at point 1 was strongly positively correlated with yellow chroma at point 2. This was the same for yellow brightness, UV chroma and UV brightness at points 1 and 2 suggesting an even distribution of colour across the yellow part feather. Yellow on the feathers was measured as it is a carotenoid-pigmented colour and therefore is subject to the trade off in use of carotenoids in the body (see Background). If a bird invests a certain amount of carotenoids into plumage colouration, we would expect to see these evenly distributed throughout the feather to get a solid band of colour. We can assume, therefore, that brighter birds with higher chroma values have a greater allocation of carotenoids invested in their feathers. Supporting this, I found that chroma and brightness are positively correlated, so that both these parameters increase together, as a result of higher carotenoid investment. There was a strong negative correlation between yellow chroma and UV chroma, whereby if yellow chroma increases, UV chroma will decrease. UV colouration is made structurally, with yellow feathers absorbing the light from the reflective white tissues that the feathers are embedded in (Shawkey

and Hill, 2005). Yellow colour displays can differ in appearance depending on both the amount of carotenoid deposited in the feather, as well as the variation in the structural colour. Shawkey and Hill (2005) found that more striking displays had high levels of carotenoids that relied heavily on the white structural colouration underneath in order to shine. One would therefore expect to find that as yellow chroma increases, UV chroma would also increase, whilst the opposite was found in this study. However, yellow brightness and UV brightness were found to be strongly positively correlated with one another, supporting the idea that having high levels of both concurrently is the most striking. The results also show that as yellow chroma increases, UV brightness decreases, and as yellow brightness increases, UV chroma decreases. These colour measures are linked by the single curve of total spectral reflectance, but further research is needed to explain these relationships.

Several studies have supported the Hamilton and Zuk hypothesis (1982) showing males with more developed sexual ornamentation have a higher resistance to parasites. Horak et al. (2004) found that greenfinches infected with coccidia had reduced expression of plumage colouration. This has also been found in house finches, *Carpodacus mexicanus*, (Hill and Brawner, 1998; Thompson et al., 1997; Nolen et al., 1998) and great tits (Horak et al., 2001). It has therefore been established that intestinal parasites, in particular coccidia, inhibit the absorption of carotenoids. The results of this project indicate that there is no link between intestinal parasite status and plumage colour and thus no effect of intestinal parasites on carotenoid absorption. All the studies that have found a relationship, have looked at birds through a moult cycle, during which time they are absorbing and depositing carotenoids in their feathers. I measured feather colour at time points independent of moult and there is little reason to assume that current IPS is related to status during last moult. In studies that investigated haemoparasites and feather mites, greenfinch plumage colour was negatively correlated with parasite load (Merila et al., 1999; Harper, 1999). I found that adult male greenfinches with brighter yellow tails were less likely to have died from trichomonas and this is consistent with the hypothesis that ornamental tail colouration acts as an honest signal of disease status. Studies have also looked at the effect of plumage colouration on immune response, finding that colour may function as an honest indicator of the ability to resist infection (Lindström and Lundström, 2000; Saks et al., 2003; Saino et al., 2003; Saino et al., 2000; McGraw and Ardia, 2003; Hill and Farmer, 2004).

There has also been research that does not support Hamilton and Zuk (1982), finding that parasite load is positively correlated with plumage colouration. Horak et al. (2001) found that adult male great tits that were infected with haemoparasites had brighter hues in their ventral yellow

pigmentation than uninfected birds. This was attributed to the fact that the infection could be at a chronic level and only a small amount of immune response would keep it at bay. Those birds unable to do this would have been filtered out at an earlier life stage. Only one study is known to me that has similarly investigated the links between plumage colour and susceptibility to infectious disease. This is a study on common redpolls, *Carduelis flammea*, by Van Oort and Dawson (2005), which have red carotenoid plumage patches on their breast and rump. There is variation between the size and colour of the patches, with adult males having more developed ornamentation. Dead redpolls were collected in British Columbia in the winter of 2002, when there was a severe salmonella outbreak. Any birds that were found to have died from causes other than salmonella were not used in the study, which in my project, would have been used as controls. Van Oort and Dawson (2005) instead used live birds as controls, assuming them to be healthy. Feathers were collected from the birds for colour analysis and the results showed that among adult male redpolls, carotenoids ornamentation was negatively correlated with probability of survival. My findings concur with those of Van Oort and Dawson (2005) as I found that yellow chroma in greenfinches was positively associated with disease status. I also found that higher values of yellow brightness were found in birds that had died of salmonella. Van Oort and Dawson (2005) justify their findings by suggesting that adult males with more striking plumage are dominant over males with less developed ornamentation. Indeed in greenfinches, both males and females display their yellow plumage during social interactions (reviewed in Lindström and Lundström, 2000) showing that it is used as an indication of social status, as well as female mate choice. Dominant males have greater access to feeding stations and therefore greater exposure to the risk of contracting a disease (Van Oort and Dawson, 2005). Adult male redpolls made up 42% of the sample of dead birds in Van Oort and Dawson's (2005) study and in my study adult male greenfinches made up 59% of the diseased sample. As I have already said, this is unlikely to be due to sampling bias and can be explained by the fact that adult males are dominant over females and younger birds at feeding stations.

Van Oort and Dawson (2005) used redness as their colour measurement by subjectively categorising the observed colour into "little or no redness", "pink" or "bright red". They also took high resolution photographs and by scanning the photos into Adobe Photoshop 6.0, they could measure the redness of the patches by getting values of hue and chroma. In my study I used a spectrometer which meant that the accuracy of the colour measurements was much higher than in Van Oort and Dawson's (2005) study. They did, however, measure the size of the red patches which I did not do in this project due to time and capacity constraints. This would have been useful, as a larger patch may

require more carotenoid investment. Van Oort and Dawson (2005) only carried out post-mortem examinations on 10 of their 79 dead redpolls. These 10 birds were all found to have salmonella and therefore, because of the severe outbreak at the time, all the dead birds were assumed to have salmonella. All of my dead greenfinches had post-mortem examinations carried out on them and not all were found to have died of the same disease and some were found to have died of trauma. I therefore think that the assumption that Van Oort and Dawson (2005) that all their dead birds died of salmonella is rather risky. The results of my study and the others summarised here contradict those that support the Hamilton and Zuk hypothesis. Further work needs to be done, which takes into account the dominance of adult birds at sights of disease risk to understand the underlying mechanisms of colour that are at work here.

My results present some interesting findings in terms of sexual selection and honest signalling. The variation that I found in adult male colouring shows that this is a sexually selected trait and that variation within this trait is noticed by females who are looking for a mate. My research does agree with the hypothesis that brighter birds were less likely to have a disease, but only in the case of trichomonas. Trichomonas is an emergent infectious disease and therefore it is a highly virulent novel pathogen to greenfinch populations. Any birds that have a superior immune system will benefit greatly from the ability to fight this disease compared to those whose immune system is not as reactive. Adult males were disproportionately represented in the sample of dead birds and as aforementioned, this may be due to their greater exposure to disease ridden feeding areas. If adult males are more affected by trichomonas, for whatever reason, then this could lead to a sex imbalance within a population. This could be detrimental to the breeding success of the population, especially as greenfinches are largely monogamous (Lindström and Lundström, 2000).

Salmonella has been in bird populations for some time and therefore is not a novel pathogen. Several generations of greenfinch populations would have been exposed to this pathogen, allowing time for a certain amount of immunity to have built up. Females would have looked for honest signals associated with disease status, such as carotenoid plumage colouration and therefore the immune system of the certain individuals would have been genetically improved through female mate choice. One would expect that if this were the case, brighter birds would be more likely to survive, but this study found the opposite. The birds who were suffering most from the disease were those that were brightly coloured, with high levels of chroma in their carotenoid pigmentation. This contrasts to the findings for trichomonas. Trichomonas is an emerging infectious disease, whereas salmonella is an infectious disease that has been present in populations for some time. It may be

that an emerging infectious disease requires a stronger immune response than an infectious disease and therefore it is more important to use carotenoids for their immune system and antioxidant roles. If tail ornaments are an honest signalling device, it may be that they are specific to various pathogens and hence the conflicting results found in this study. More work is needed to compare the results of carotenoid tail colour interacting with infectious and emerging infectious disease before we can be sure that it is an honest signalling device.

In terms of conservation of garden birds, one of the aims of the Garden Bird Health Initiative (see Background) is to identify what ecological factors are associated with higher disease risk. From my findings I would suggest that dominant adult males, with highly developed carotenoid plumage, are most at risk and therefore the act of keeping feeding stations and the area around them clean may be key to increasing the survival success of these birds. This is what the GBHi is trying to promote through the assistance of the public and volunteers who participate in the monitoring network. Currently feeding practice in the UK may be detrimental to the species by removing the higher quality adult males in terms of mate preference. This could well be disrupting the natural process of sexual selection in greenfinches, leading to less viable populations.

## References

- Aguilera E & Amat JA (2007) Carotenoids, immune response and the expression of sexual ornaments in male greenfinches (*Carduelis chloris*). *Naturwissenschaften* **94**: 895-902
- Andersson M (1994) Sexual Selection. *Princeton University Press, New Jersey*.
- Ball AP (1982) Notes on Infectious Diseases. *Churchill Livingstone, New York*.
- Bertrand S, Faivre B & Sorci G (2006) Do carotenoid-based sexual traits signal the availability of non-pigmentary antioxidants? *J Exp Biol* **209**: 4414-4419
- Biard C, Surai PF & Möller AP (2005) Effects of carotenoid availability during laying on reproduction in the blue tit. *Oecologia* **144**: 32-44
- Biard C, Surai PF & Möller AP (2006) Carotenoid availability in diet and phenotype of blue and great tit nestlings. *J Exp Biol* **209**:1004-1015
- Birdlife International (2004) *Carduelis chloris* In: IUCN 2007. *2007 IUCN Red List of Threatened Species*
- Blount JD, Houston DC, Surai PF & Möller AP (2004) Egg laying capacity is limited by carotenoid pigment availability in wild gulls *Larus fuscus*. *Proc. R. Soc. Lond. B (Suppl.)* **271**: S79-S81
- Blount JD, Surai PF, Nager RG, Houston DC, Möller AP, Trewby ML & Kennedy MW (2001) Carotenoids and egg quality in the lesser black-backed gull *Larus fuscus*: a supplemental feeding study of maternal effects. *Proc. R. Soc. Lond. B* **269**:29-36
- Boal CW, Mannan RW & Hudelson KS (1998) Trichomoniasis in Cooper's Hawks from Arizona. *Journal of Wildlife Diseases* **34(3)**: 590-593
- Constani D & Möller AP (2008) Carotenoids are minor antioxidants for birds. *Functional Ecology* **22**: 367-370
- Constanti D, Casagrande S, De Filippis S, Brambilla G, Fanfani A, Tagliavini J & Dell'Omo G (2006) Correlates of oxidative stress in wild kestrel nestlings (*Falco tinnunculus*). *J Comp Physiol B* **176**: 329-337
- Constanti D, Fanfani A & Dell'Omo G (2007) Carotenoid availability does not limit the capacity of nestling kestrels (*Falco tinnunculus*) to cope with oxidative stress. *J Exp Biol* **210**: 1238-1244

Crawley MJ (2007) *The R Book*. John Wiley and Sons, Chichester

Dufva R & Allander K (1995) Intraspecific Variation in Plumage Colouration Reflects Immune Response in Great Tit (*Parus major*) males. *Functional Ecology* **9(5)**: 785-789

Evans MR & Thomas ALR (1992) The aerodynamic and mechanical effects of elongated tails in the scarlet-tufted malachite sunbird: Measuring the costs of a handicap. *Animal Behaviour* **43(2)**: 337-347

Ewen JG, Surai P, Stradi R, Moller AP, Vittorio B, Griffiths R & Armstrong DP (2006a) Carotenoids, colour and conservation in an endangered passerine, the hihi or stitchbird (*Notiomystis cincta*). *Animal Conservation* **9**: 229-235.

Ewen JG, Thorogood R, Karadas F & Cassey P (2008) Condition dependence of nestling mouth colour and the effect of supplementing carotenoids on parental behaviour in the hihi (*Notiomystis cincta*). *Oecologia* DOI: 10.1007/s00442-008-1073-3

Ewen JG, Thorogood R, Karadas F, Pappas AC & Surai PF (2006b) Influences of carotenoid supplementation on the integrated antioxidant system of a free living endangered passerine, the hihi (*Notiomystis cincta*). *Comparative Biochemistry and Physiology* **143**: 149-154

Ewen JG, Thorogood R, Nicol C, Armstrong DP & Alley M (2007) Salmonella Typhimurium in Hihi, New Zealand. *Emerging Infectious Diseases* **13(5)**: 788-789

Fischer JR, Stallknecht DE, Luttrell MP, Dhondt AA & Converse KA (1997) *Mycoplasmal Conjunctivitis* in Wild Songbirds: The Spread of a New Contagious Disease in a Mobile Host Population. *Emerging Infectious Diseases* **3(2)**

Fitze PS & Tschirren B (2006) No evidence for survival selection on carotenoid-based nestling colouration in great tits (*Parus major*). *J Evol Biol* **19**: 618-624

GBHi, Best Practice Guidelines. <http://www.ufaw.org.uk/gbhi.php>

GBHi, Salmonella advice sheet. <http://www.ufaw.org.uk/gbhi.php>

GBHi, Trichomonas advice sheet. <http://www.ufaw.org.uk/gbhi.php>

Grant D, Todd PA & Pennycott T (2007) Monitoring wild greenfinch (*Carduelis chloris*) for *Salmonella enteric typhimurium*. *Ecol Res* **22**: 571-574

- Hamilton WD & Zuk M (1982) Heritable True Fitness and Bright Birds: A Role for Parasites? *Science* **218(4570)**: 384-387
- Harper DGC (1999) Feather mites, pectoral muscle condition, wing length and plumage colouration of passerines. *Animal Behaviour* **58(3)**: 553-562
- Hartley RC & Kennedy MW (2004) Are carotenoids a red herring in sexual display? *Trends in Ecology and Evolution* **19(7)**: 353-354
- Hill GE & Brawner WR (1998) Melanin-Based Plumage Colouration in the House Finch is Unaffected by Coccidial Infection. *Pro. Biol. Sc.* **265(1401)**: 1105-1109
- Hill GE (2006) Bird Colouration: Mechanism and Measurements Vol. 1. *Harvard University Press, USA*
- Hill GE (2006b) Bird Colouration: Function and Evolution Vol. 2. *Harvard University Press, USA*
- Hill GE (1991) Plumage colouration is a sexually selected indicator of male quality. *Nature* **350**: 337-339
- Hill GE and Farmer KL (2005) Carotenoid-based plumage colouration predicts resistance to a novel parasite in the house finch. *Naturwissenschaften* **92**: 30-34
- Hill GE, Montgomerie R, Inouye CY & Dale J (1994) Influence of Dietary Carotenoids on Plasma and Plumage colour in the House Finch: Intra- and Intersexual Variation. *Functional Ecology* **8(3)**: 343-350
- Hill GE, Nolan PM & Stoehr AM (1999) Pairing success relative to male plumage redness and pigment symmetry in the house finch: temporal and geographic constancy. *Behavioural Ecology* **10(1)**: 48-53
- Horak P, Ots I, Vellau H, Spottiswoode C & Möller AP (2001) Carotenoid-based plumage colouration reflects haemoparasites infection and local survival in breeding great tits. *Oecologia* **126**: 166-173
- Horak P, Saks L, Karu U, Ots I, Surai PF & McGraw KJ (2004) How coccidian parasites affect health and appearance of greenfinches. *Journal of Animal Ecology* **73**: 935-947
- Horak P, Vellau H, Ots I & Möller AP (2000) Growth conditions affect carotenoid-based plumage colouration of great tit nestlings. *Naturwissenschaften* **87**: 460-464
- Horak P, Zilmer M, Saks L, Ots I, Karu U & Zilmer K (2006) Antioxidant protection, carotenoids and the costs of immune challenge in greenfinches. *J Exp Biol* **209**: 4329-4338

- Hunt S, Kilner RM, Langmore NE & Bennett ATD (2003) Conspicuous, ultraviolet rich mouth colours in begging chicks. *Proc. R. Soc. Lond. B (Suppl.)* **270**: S25-S28
- Jennions MD, Möller AP and Petrie M (2001) Sexually Selected Traits and Adult Survival: A Meta-Analysis. *The Quarterly Review of Biology* **76(1)**: 3-36
- Johnsen A, Delhey K, Andersson S & Kempenaers B (2003) Plumage colour in nestling blue tits: sexual dichromatism, condition dependence and genetic effects. *Proc. R. Soc. Lond. B* **270**: 1263-1270
- Kotiaho JS (2001) Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol Rev* **76**: 365-376
- Lessells CM & Boag PT (1987) Unrepeatable repeatabilities: A common mistake. *Auk* **104**: 116-121
- Lindström K & Lundström J (2000) Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus clearance rate. *Behav. Ecol. Sociobiol.* **48**: 44-51
- Lindström K (2004) Social status in relation to Sindbis virus infection clearance in greenfinches. *Behav. Ecol. Sociobiol.* **55**: 236-241
- Lindström K, Krakower D, Lundström J & Silverin B (2001) The effect of testosterone on a viral infection in greenfinches (*Carduelis chloris*): an experimental test of the immunocompetence-handicap hypothesis. *Proc. R. Soc. Lond.* **268**: 207-211
- McGraw KJ & Ardia DR (2003) Carotenoids, Immunocompetence, and the Information Content of Sexual Colours: An Experimental Test. *The American Naturalist* **162(6)**: 705-712
- McGraw KJ (2004) Colourful songbirds metabolize carotenoids at the integument. *J Avian Biol* **35**: 471-476
- McGraw KJ, Adkins-Regan E & Parker RS (2005) Maternally derived carotenoid pigments affect offspring survival, sex ratio and sexual attractiveness in a colourful songbird. *Naturwissenschaften* **92**: 375-380
- McGraw KL & Hill GE ( ) Differential effects of endoparasitism on the expression of carotenoids- and melanin-based ornamental colouration. *Proc. R. Soc. Lond. B* **267**: 1525-1531
- McKeon T, Dunsmore J & Raidal SR (1997) *Trichomonas gallinae* in budgerigars and colombid birds in Perth, Western Australia. *Aust Vet J* **75(9)**: 652-655

- Merila J, Sheldon BC & Lindström K (1999) Plumage brightness in relation to haematozoan infections in the greenfinch (*Carduelis chloris*): Bright males are a good bet. *Ecoscience* **6(1)**: 12-18
- Möller AP (1988) Female choice selects for male sexual tail ornaments in the monogamous swallow. *Nature* **332**: 640-642
- Möller AP (1990) Parasites and sexual selection: Current status of the Hamilton and Zuk hypothesis. *J Evol Biol* **3**: 319-328
- Möller AP, Christe P & Lux E (1999) Parasitism, Host Immune Function, and Sexual Selection. *The Quarterly Review of Biology* **74(1)**: 3-20
- Nolan PM, Hill GE & Stoehr AM (1998) Sex, size, and plumage redness predict house finch survival in an epidemic. *Proc. R. Soc. Lond. B* **265**: 961-965
- Olsen VA & Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? *Trends in Ecology and Evolution* **13(12)**: 510-514
- Olsen VA & Owens IPF (2005) Interspecific variation in the use of carotenoid-based colouration in birds: diet, life history and phylogeny. *J Evol Biol* **18**: 1534-1546
- Richner H, Christe P & Oppliger A (1995) Paternal investment affects prevalence of malaria. *Proc. Nat. Aca. Sci. USA* **92**: 1191-1194
- Saino N, Ambrosini R, Martinelli R, Ninni P & Möller AP (2003a) Gape colouration reliably reflects immunocompetence of barn swallow (*Hirundo rustica*) nestlings. *Behavioural Ecology* **14(1)**: 16-22
- Saino N, Ferrari R, Romano M, Martinelli R & Möller AP (2003b) Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. *Proc. R. Soc. Lond.* **270**: 2485-2489
- Saino N, Ninni P, Calza S, Martinelli R, De Bernardi F & Möller AP (2000) Better red than dead: carotenoid-based mouth colouration reveals infection in barn swallow. *Proc. R. Soc. Lond.* **267**: 57-61
- Saks L, Karu U, Ots I & Horak P (2006) Do standard measures of immunocompetence reflect parasite resistance? The case of greenfinch coccidiosis. *Functional Ecology* **20**: 75-82
- Saks L, McGraw KJ & Horak P (2003) How feather colour reflects its carotenoid content. *Functional Ecology* **17**: 555-561

- Saks L, Ots I & Horak P (2003) Carotenoid-based plumage colouration of male greenfinches reflects health and immunocompetence. *Oecologia* **134**: 301-307
- Shawkey MD & Hill GE (2005) Carotenoids need structural colours to shine. *Biol. Lett.* **1**: 121-124
- Sheldon BC & Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology *Trends in Ecology and Evolution* **11(8)**: 317-321
- Thompson CW, Hillgarth N, Leu M & McClure HE (1997) High Parasite Load in House Finches (*Carpodacus mexicanus*) is Correlated with Reduced Expression of a Sexually Selected Trait. *The American Naturalist* **149(2)**: 270-294
- Thorogood R, Kilner RM, Karadas F & Ewen JG (2008) Spectral mouth colour of nestlings change with carotenoids availability. *Journal of Functional Ecology (In Press)*
- Trams EG (1969) Carotenoid transport in the plasma of the Scarlet Ibis (*Eudocimus ruber*). *Comparative Biochemistry and Physiology* **28**: 1177-1184
- Tschirren B, Fitze PS & Richner H (2003) Proximate mechanisms of variation in the carotenoid-based plumage colouration of nestling great tits (*Parus major* L.) *J Evol Biol* **16**: 91-100
- Van Oort H & Dawson RD (2005) Carotenoid ornamentation of adult male Common Redpolls predicts probability of dying in a Salmonellosis outbreak. *Functional Ecology* **19**: 822-827
- Von Schantz T, Bensch S, Grahn M, Hasselquist D & Wittzell H (1999) Good genes, Oxidative stress and Condition-Dependent Sexual Signals. *Pro. Biol. Sc.* **266(1414)**: 1-12
- Yu CY, Chu C, Chou SJ, Chao MR, Yeh CM, Lo DY, Su YC, Horng YM, Weng BC, Tsay JG & Huang KC (2008) Comparison of the Association of Age with Infection of *Salmonella* and *Salmonella enteric Serovar Typhimurium* in Pekin Ducks and Roman Geese. *Poult Sci* **87**: 1544-1549
- Zahavi A (1975) Mate selection - a selection for a handicap. *J Theor Biol* **53(1)**: 205-214

**Figures and Tables**

- Figure 2.1:** Lutein structure (Surai, 2003).....Page 13
- Figure 2.2:** Diagram showing factors that affect carotenoid absorption, including metabolic factors, dietary factors, subject factors and food form as reviewed in Surai (2003).....Page 15
- Figure 2.3:** Munsell Colour System showing hue, brightness and chroma (webvision.med.utah.edu/KallColor.html).....Page 25
- Figure 3.1:** Map of the UK showing distribution of greenfinches used in this project. All greenfinches were found throughout England and Wales.....Page 26
- Figure 3.2:** Outer right tail feather from adult male greenfinch mounted on black card for colour analysis. The circles show the standard locations where colour measures were taken across feathers from different individuals.....Page 28
- Figure 4.1:** Scatter diagrams showing correlations between yellow chroma, yellow brightness, UV chroma and UV brightness. a) YC1:YB1, b) YC1:UC1, c) YC1:UB1, d) YB1:UC1, e) YB1:UB1, f) UB1:UC1, g) YC1:YC2, h) YB1:YB2, i) UC1:UC2 and j) UB1:UB2.....Page 33
- Figure 4.2:** Comparison of yellow chroma for points 1 and 2 on feathers in New Zealand and UK trauma greenfinches.....Page 34
- Figure 4.3:** Prevalence of diseases compared between age sex groups. a) salmonella versus age; b) trichomonas versus age; c) trichomonas versus sex. The dark area represents females and the light area represents males. The widths of the bars vary depending on the number of birds in each status category.....Page 36
- Figure 4.4:** Boxplots showing colour measures against disease status. a) YC1 with respect to overall disease status; b) YB1 with respect to trichomonas status; c) YB1 with respect to salmonella status. Heavy bars represent the median colour measurements, the bottom and top of the boxes represent the 25<sup>th</sup> and 75<sup>th</sup> percentiles respectively, whiskers represent 1.5 times the interquartile range of the data, and points represent the outliers (Crawley, 2007).....Page 38

**Table 4.1:** Comparison of yellow chroma, yellow brightness, UV chroma and UV brightness at point 1 and point 2 on greenfinch feathers.....Page 31

**Table 4.2:** Results from four generalised linear models, with the parameters sex, age and habitat as explanatory variables and disease status, intestinal parasite status (IPS), salmonella status and trichomonas status as dependent variables. The table shows the values for chi squared and p from the minimal adequate models, with all having d.f. = 1. Where the variables were significant, they have been named as there is a lower p value, being <0.05. Where there were no significant variables, the p value is higher, being >0.005.....Page 35

**Table 4.3:** Results from four generalised linear models, with colour parameters YC1, YC2, YB1 and YB2 as explanatory variables and disease status, IPS, salmonella status and trichomonas status as dependent variables. The table shows the values for chi squared and p from the minimal adequate models, with all having d.f. = 1.....Page 37