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Dietary conservatism in passerines: the influences of novel odour and novel colour.
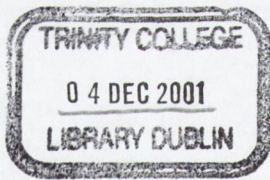
By

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This thesis is submitted in fulfilment of requirements for the degree of
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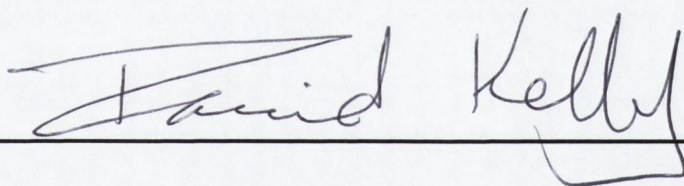
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Summary

This research was concerned with the way that passerine bird species use odour cues in isolation and in combination with colour cues to make decisions about new foods. Galliformes are known to demonstrate a disproportionately large latency to eat food with novel odour and novel colour cues. Passerine species, although possessing a smaller olfactory lobe than galliformes, are known to be able to detect odours at similar concentrations to galliformes. The behaviour of passerines towards these cues has however been poorly studied. In order to investigate the effects of environment and social structure on these behaviours, the experimental design was separated into three areas: solitary wild birds (birds which maintain winter territories), social wild birds (birds which form large feeding flocks in winter) and laboratory-held birds. The laboratory studies were envisaged to allow investigation of observed wild-type behaviour under controlled conditions.

The wild studies presented the birds with four treatments of food in a food choice arrangement. The laboratory studies replicated the design of previous studies involving galliformes, and so presented these treatments in isolation. The four treatment groups were: control, novel odour, novel colour and novel odour and novel colour in combination. A fourth experimental group was added to the data to investigate a potentially confounding effect of familiar odour. This group replicated the solitary wild group study using chicks. There was a large variation in the responses of individuals to the stimuli, but all four groups of birds showed the same general trends. There was an increased latency to incorporate familiar foods of novel colour, and an indication that the novel odour cues were being attended to, when accompanied by a novel colour.

Analysis of the results indicated that there are two discrete components to the process of dietary incorporation. The first component considers the approach of the bird to the novel food (neophobia) and the second component concerns the consumption of the food (dietary conservatism). These two components varied in relative magnitude when considering the different species involved in the study. Detailed recording of the choices of the birds in the solitary wild bird study revealed an added degree of complexity to the process of dietary incorporation, and suggested that it is comprised of at least four stages.

Further consideration of the processes of dietary conservatism and neophobia proposed important roles for them in both the evolution of aposematic animals, and also the polymorphism of established aposemes. There may also be commercial applications of these processes in the protection of stored grain from avian pests.

Acknowledgements

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Chapter 1

General Introduction

1.1 Introduction:

In order to fully appreciate the questions posed by this study, it is necessary to review experimental findings in a number of apparently disparate areas. These areas combine to provide an holistic appreciation of the subject. It is necessary to consider the question of whether birds have an olfactory sense (section 1.2) in order to understand their reactions to odour cues. It is also necessary to define the key behavioural processes of neophobia (section 1.3) and dietary conservatism (section 1.5). Natural predator-prey interactions are also fundamental to an understanding of the subject. A number of animals gain protection by advertising their unprofitability to potential predators (aposematism – section 1.6). It is important to understand what learning and memory processes are at work here too (section 1.7). Other prey species have adopted strategies that are determined by their population densities (frequency-dependent selection – section 1.4). The predators themselves also require some introduction, as they are less frequently studied in this area than other avian families (the importance of passerines – section 1.8). This introduction is then followed by a brief description of the aims of the experiments conducted (experimental plan – section 1.9).

1.2 Avian Olfaction:

1.2.1 Behavioural studies

The sense of smell in birds is not a sense that is immediately obvious. With the exception of kiwis, birds do not give the impression of 'sniffing' their surroundings, and there is even some doubt as to whether the sniffing action of kiwis is actually involved in olfaction (Wenzel 1971). However, there is a growing body of work to indicate that olfaction plays an important role in the lives of many bird species (for review see Roper 1999). Behavioural studies have demonstrated olfactory navigation in the homing (feral) pigeon, *Columba livia* (Papi 1991; Papi 1995); olfactory detection of food in vultures (Stager 1964; Gomez *et al.* 1994) and certain seabirds (Grubb 1972; Hutchison & Wenzel 1980; Hutchison *et al.* 1984; Jouventin & Robin 1984; Wenzel 1985; Wenzel 1986; Lequette *et al.* 1989; Clark & Shah 1992; Verheyden & Jouventin 1994); responses to warning odours produced by aposematic insects (Guilford *et al.* 1987; Rothschild & Moore 1987; Marples & Roper 1996); selection of nesting materials by odour (Clark & Mason

1987); an olfactory memory in both the feral pigeon (Honey *et al.* 1990) and domestic chicks (*Gallus gallus domesticus*) (Jones & Faure 1982; Jones & Gentle 1985; Jones & Carmichael 1999a); and the potential for protection of food crops from wild birds by odorous, non-toxic avian aversants (Järvi & Wiklund 1984; Crocker & Perry 1990; Mason & Otis 1990; Gill *et al.* 1994; Watkins *et al.* 1995). Chapter 5 covers this last group in more detail.

1.2.2 *Physiological studies*

Edinger (1908) suggested initially that the size of the olfactory bulb in comparison with the rest of the brain could be important in predicting which species will rely on olfaction. Cobb (1960a,b) defined this relative olfactory bulb size (ROBS) index as being the ratio of the greatest diameter of the olfactory bulb to that of the ipsilateral cerebral hemisphere. This resulted in over 100 species from over 20 different orders being measured (Cobb 1960a, b; Bang 1968; Bang & Cobb 1968; Bang 1971; Goldsmith & Goldsmith 1982; Bang & Wenzel 1985; Ioalè & Papi 1989). ROBS scores were shown to be high in a number of seabird species, intermediate in domestic pigeons and galliformes (domestic fowl and game birds), and low in passerine species (finches, sparrows, thrushes and warblers). A more recent system (Healy & Guilford 1990) using a hierarchical-multiple-regression analysis, which controls for the body weight of birds has shown a strong correlation between nocturnality and a high ROBS score. From the data collected on tube-nosed seabirds (*Procellaridae*), including albatrosses, shearwaters and petrels they found a slight, but significant, difference between nocturnal and diurnal species. As a group, these seabirds still had relatively high ROBS scores (Bang & Cobb 1968), but the scores for the diurnal seabirds were lower. Similarly, the finches studied (*Fringillidae*) showed the same trend, but the group as a whole had relatively low ROBS scores (Bang & Cobb 1968). The increase in the olfactory sense appears to have evolved to 'compensate' for the limitations of the sense of sight under low light conditions (Healy & Guilford 1990).

1.3 <u>Neophobia</u>:

1.3.1   Definition

Neophobia is, simply put, a fear of novelty. The term was first coined by Barnett (1958) during studies with rats, although 'new object reaction' was demonstrated earlier by Chitty and Shorten (1946) also working on rats. Barnett (1958) found that by changing the position and shape of a food container in a familiar cage, all of the five wild rats he tested stopped eating for at least seven hours. Three of these didn't eat for more than 24 hours. Barnett noted that there was a 'great individual variation' in the reactions of these wild rats. Two other groups of wild rats reduced their food intake on the first day that additional empty food containers were added to their cages, but their responses were much less dramatic than the first group. When Barnett conducted the same tests on tame albino and hooded rats he recorded very different results. Although these animals interrupted their feeding patterns to explore the new objects, their daily food intake was unaffected. Barnett concluded, "only wild rats display neophobia in any substantial form". Subsequent reanalysis of this work (Mitchell 1976) has shown that domestic varieties of rats do show lower, but measurable, levels of neophobia.

A recent review of neophobia (Brigham & Sibly 1999) suggested an operational definition as "the initial avoidance of novel objects in an otherwise familiar environment". Here, the term 'objects' may include food. This is important, as the behavioural literature tends to consider this specialised case most frequently. The psychology literature occasionally refers to food neophobia as 'hyponeophagia' (Shephard & Broadhurst 1981; Shephard & Broadhurst 1982), but psychological studies tend to focus on the responses and behaviours of the individual, and so frequently deal with non-food objects (Shorten 1954; Cowan & Barnett 1975; Cowan 1976; Corey 1978; Misslin 1982; Meshkova *et al.* 1985; Wallace 1988). The use of the word "initial" in Brigham and Sibly's definition is also important, because the avoidance will decrease with time as the new objects lose their novelty (Barnett 1958).

1.3.2   Original experiments

Coppinger (1969, 1970) is often credited with being the first person to demonstrate neophobia in birds. He studied the responses of captive blue jays (*Cyanocitta cristata*), common grackles (*Quiscalus quiscula*) and red-winged blackbirds (*Agelaius phoeniceus*) towards neo-tropical butterflies (i.e. a novel food source). He showed that both hand-raised

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and wild-caught birds often showed fearful responses to these novel prey: the blue jays retreated to the farthest corner of their cages from the butterfly, and sometimes raised their crests or gave alarm calls. The birds were described as showing an active rejection of their novel food item. The reactions of the birds were demonstrated to be the result of experience. Two groups of birds responded quite differently to two similarly sized butterflies (Coppinger 1970). *Anartia jatrophae* is a brown and white butterfly, whereas *A. amalthea* is a bright red and black butterfly. Most naïve birds exposed to *A. jatrophae* 'habituated' to it, i.e. they ceased to show fearful reactions to it during the experimental period (18 presentations), whereas naïve birds exposed to *A. amalthea* did not habituate. Birds that had habituated to *A. jatrophae* were then tested with *A. amalthea*. Six of the nine birds that had attacked *A. jatrophae* attacked *A. amalthea* during their 18-presentation period. Birds that had failed to attack *A. jatrophae* never attacked *A. amalthea*. Birds from this test that were eating both butterflies were then tested with a third species (*Protogonius hippona*). *P. hippona* showed differences in size, shape, colour and pattern from the previously experienced butterflies. Only two of the seven birds that had reached this stage attacked these butterflies without hesitation.

It appears that the novelty of these butterflies was perceived in a number of different ways. Colour, shape, size and pattern all appear to be important in an assessment of familiarity. Birds with experience of a few of these characters beforehand could reduce their latencies to contact what naïve birds would consider to be highly novel prey. Similar results with chimpanzees had been previously achieved (Menzel 1963). Coppinger proposed that the birds were not showing innate responses to particular colours or patterns, but to novelty itself.

1.3.3 Innate aversions

Several studies after Coppinger's work (Coppinger 1969; Coppinger 1970) have shown that some predators are reluctant to sample novel, conspicuously coloured natural (Wiklund & Järvi 1982; Brunner & Coman 1983; Wiklund & Sillén-Tullberg 1985; Madsen 1987) or artificial (Smith 1975; Smith 1977; Schuler 1982; Caldwell & Rubinoff 1983; Schuler & Hesse 1985; Lindström *et al.* 1999a) prey (review in Schuler & Roper 1992). There are particular colours and patterns that birds appear to avoid innately.

A number of authors have found that red prey appears to be innately aversive to avian predators (Sillén-Tullberg 1985; Roper 1990; Mastrota & Mench 1995). Roper

(1990) found that red prey was more aversive to naïve chicks than brown prey, on both red and brown backgrounds. He concluded "specific sign-stimuli are aversive in themselves, regardless of background colour". A similar innate aversion has been found to a yellow and black striped pattern (Schuler 1982; Schuler & Hesse 1985; Roper & Cook 1989; Lindström et al. 1999a). Roper and Cook (1989) used a number of colour combinations (yellow and black, red and black, and red and yellow) and patterns (plain colour, 'bicoloured', and striped) in an attempt to look at the contrast effects in more detail. They found that chicks were only strongly averse to plain black and black and yellow striped prey. Guilford (1990) suggested that striped patterns were effective deterrents to predators since they contained internal colour-contrast boundaries. However, this does not explain why Roper and Cook found no strong avoidance of red and black striped, and red and yellow striped prey. It is interesting to reflect that an object that had two equal yellow and black halves was not significantly aversive to the chicks, whereas one with multiple yellow and black stripes was (Roper & Cook 1989). These innate aversions are not insurmountable. Roper and Cook found that some chicks, after repeated exposure, overcame their aversion to the yellow and black striped prey, and consumed them at a high rate.

The processes underlying these innate aversions are not yet understood. Although one might suppose that many insects are exploiting the innate aversions of their predators, there is still something of a 'chicken and egg' question to the subject. Have insects evolved owing to a random aversion of their predators, or have the predators become 'genetically programmed' to avoid frequently encountered unprofitable colours and patterns?

1.3.4 Effects of experience

A growing body of work indicates that neophobic responses can be reduced significantly by previous exposure to novel stimuli, providing these exposures were not unpleasant (Coppinger 1969; Coppinger 1970; Schlenoff 1984; Jones 1986; Mastrota & Mench 1995). Marples and Speed (unpublished data) compared duration and variety of experience on the reactions of chicks to novel food. They found that four exposures to one novel colour of food (red or blue) reduced latency to attack novel black food just as much as four single exposures to four different colours (red, orange, yellow and green in any sequence). More recent work has suggested that neophobia to a novel colour in chicks can be deactivated by pre-exposure to any other colour for just one to two minutes (M. Quinlan unpublished data). However, if neophobia is reduced with respect to one aspect of novelty,

there is no evidence that this is true for all other aspects simultaneously. Further studies are required to know, for example, whether the neophobia induced by a novel food type (e.g. grain instead of pastry) is deactivated by prolonged exposure to novel colours.

Another measure of experience is the age of an animal. One could argue that like naïve predators, younger individuals would show greater hesitancy to attack novel foods, since they have no knowledge of their safety. On the other hand, a very young bird might not be aware of the defences of aposematic insects, and might investigate new foods simply out of curiosity. The literature is not at all clear as to the effect of age on neophobic responses. Jones and Carmichael (1999b) found that older chicks (10 days old) showed greater latencies to peck at bunches of coloured string than did younger chicks (2 days old). However, a comparison of other experiments by Jones and his colleagues provides contrary information. Jones (1986) looked at the neophobia of chicks towards novel coloured food (blue). He found that from a sample of 38 'T line' chicks (a cross between 'Rhode Island Red' and 'Light Sussex') the average time taken to eat blue food was 515s (+/- 72s). A similar experiment (Jones & Andrew 1992) with older capons and roosters (22 weeks) of a 'Warren SSL' breed (a cross between 'Rhode Island Red' and 'Rhode Island White') showed that on average roosters were more neophobic than capons; latencies to contact novel coloured food (blue) were 398s (+/- 86s) and 115s (+/- 64s) respectively. However, both of these values are lower than the average chick latency from the earlier experiment (Jones 1986). Mastrota and Mench (1995) found no differences between the neophobic responses of bobwhite quail (*Colinus virginianus*) to blue food between the ages of 11 and 31 weeks. This comparison was chosen because the quail become sexually mature between these ages. It would appear unwise to draw firm conclusions from these apparently contradictory results.

Environmental experience appears to be an important consideration too. Wild and laboratory-held animals tend to differ in their general responses to novel coloured food (review in Marples and Kelly (1999)). Laboratory-held birds tend to show shorter latencies to eat novel coloured food than do wild birds. Barnett (1958) showed a similar effect in his rat study. When food was presented to the test animals in a new container, and in a new location, wild-caught rats showed much greater interruption to their feeding habits than did tame albino or tame hooded rats. Laboratory-held birds are probably unlikely to be exposed to a greater variety of stimuli than wild birds but, as they do not have the option to avoid them, are more likely to habituate to them than their wild counterparts.

1.3.5 Interaction of sensory cues

Multimodal signals (i.e. simultaneous signals in different sensory modalities) appear to produce greater avoidance than their component unimodal signals alone (Marples *et al.* 1994; Marples & Roper 1996; Rowe & Guilford 1996, 1999b). The various novel aspects of these complex signals appear to interact, producing a greater combined aversion than would be predicted by simple addition. This synergistic effect was first demonstrated in the laboratory with domestic chicks (Marples & Roper 1996). Marples and Roper (1996) presented chicks with starter crumbs in the presence and absence of a novel odour. They showed there was no significant difference in the latencies of the birds to eat these two treatments. However, chicks presented with novel coloured starter crumbs (green or blue) in the presence of certain novel odours (2-methoxy-3-sec-butyl pyrazine, 2-methoxy-3-isobutyl pyrazine, almond essence and vanilla essence) showed a significantly increased latency to eat these compared to the same crumbs without the novel odour. A similar result was found when chicks were tested for avoidance of coloured water instead of food (Marples & Roper 1996). Rowe and Guilford (1996) looked at the preferences of chicks to food of different colours in the presence and absence of a pyrazine (2-isobutyl-3-methoxy pyrazine). They found that chicks showed a bias towards green food over both red and yellow food in the presence of the pyrazine, where there was no bias without pyrazine. These biases appeared to wane over time. Rowe and Guilford suggested that in order to maintain such a bias, a prey species would need to acquire unpalatability. Recent work by Rowe and Guilford (1999b) has shown that combinations of novel sound and novel colour have a similar synergistic effect.

Rowe (1999a) refers to the synergistic effect of colour and odour in terms of the odour potentiating the aversion to the novel colour. Flavour has been demonstrated to potentiate the conditioning of colour cues in pigeons (Clarke *et al.* 1979; Lett 1980), quail (*Coturnix coturnix japonicus*) (Lett 1980) and hawks (Brett *et al.* 1976).

Some stimuli are remembered as being more important indicators of information, i.e. they overshadow other stimuli. In an experiment which tested the responses of chicks to colours and odours, Roper and Marples (1997a) discovered that almond and vanilla odour overshadowed colour as a cue in taste-avoidance learning. Once chicks had learned that water with an almond or vanilla odour tasted bad (flavoured with quinine), they were reluctant to sample it. However, they would readily drink other water that did not smell the same, irrespective of its colour.

1.3.6   Aggregation effects

As well as interactions between predators, the interaction of the prey themselves may influence the responses of their predators. Aggregations of aposematic prey are known to produce quicker and stronger aversions in predators than individual prey (Gagliardo & Guilford 1993; Gamberale & Tullberg 1996; Marples & Kelly 1999). However, aggregations of palatable prey do not appear to be aversive (Gamberale & Tullberg 1998), even if they are conspicuously coloured (Lindström *et al.* 1999a). On the other hand, the deterrent effect of aggregated unpalatable prey does not necessarily need conspicuous coloration (Gittleman & Harvey 1980; Gittleman *et al.* 1980; Alatalo & Mappes 1996). Indeed Speed & Marples (in press) have even shown a deterrent effect for aggregations of palatable and cryptic prey.

The fact that aposematic prey benefit from aggregations is not contested. However, the evolution of this protection, and also the role of aggregation in the evolution of aposematism are subjects still under debate (review in Guilford 1990).

1.3.7   A genetic basis?

The individual variation of neophobic reactions observed in populations (Chitty 1954; Barnett 1958) may in part be owing to genetic factors. Jones (1986) added weight to this argument when he compared two strains of domestic chicks on their neophobia towards blue food. He found that a 'T line' strain (a cross between Rhode Island Red and Light Sussex breeds) had a mean latency to eat of 516s (+/- 72s), whereas an 'S line' strain (White Leghorn) had a mean latency of 327s (+/- 66s). A Mann-Whitney U test between these two groups gave a significant result ($p < 0.02$). One might expect all naïve predators of the same species to behave similarly. Clearly this is not the case.

Marples and Brakefield (1995) found that they could breed a bird species (quail) with increased ('SLOW') or reduced ('FAST') neophobic reactions to a novel prey item (two-spot ladybirds *Adalia bipunctata*) by selecting from parents with the most extreme responses to this prey item. By the third generation (F3), there was a highly significant difference between the latencies of the 'FAST' and 'SLOW' lines to eat novel prey. These F3 generations were also assessed as to their general 'fearfulness'. This test added novel objects close to familiar food. There was found to be no correlation between the selection line ('FAST' or 'SLOW') and the speed of eating in these tests. This strongly suggests two different components of neophobic reactions, one relating to prey (the main test of the experiment) and one relating to environment (the general fearfulness test). Marples and

Brakefield were careful to point out that neophobic reactions to food and the general boldness or fearfulness of an individual should be considered separately.

1.3.8   *Previous work on passerines*

A number of authors have looked at the neophobic effects of novel colours of food on the feeding behaviour of wild or wild-caught passerine species (Ridsdale & Granett 1969; Brunner & Coman 1983; Pawlina & Proulx 1996; Marples *et al.* 1998; Jetz *et al.* 2001). All of them found that a novel colour of a familiar food reduces feeding. While previous studies have looked at the neophobic responses of the zebra finch, *Taeniopygia guttata* (Rabinowitch 1969; Sillén-Tullberg 1985; Coleman & Mellgren 1994; Coleman & Mellgren 1997), and the blackbird, *Turdus merula* and robin, *Erithacus rubecula* (Marples *et al.* 1998) to food of novel colour, no work appears to have been done on odour perception in these species.

Rabinowitch (1969) looked at the seed preferences of zebra finches, and the role of experience in the development of these preferences. He found that the critical period for acquiring food preferences was the fledgling period (3rd and 4th weeks after hatching), and that the preferences made during this time were maintained for at least four months, even when the birds were allowed to feed freely from a seed mixture.

Sillén-Tullberg (1985) presented pairs of zebra finches with novel larvae of the seed bug *Lygaeus equestris*. The birds were presented with both the normal red form and a mutant grey form of the larvae, against matching and contrasting backgrounds (i.e. grey and red). She found that grey larvae were attacked more frequently irrespective of the background colour. She concluded that the zebra finches were rejecting the red larvae on colour alone, and not allowing the contrast (or lack of it) with the background to affect their choice.

Marples *et al.* (1998) looked at the effects of neophobia and dietary conservatism (see below). They offered blackbirds and robins novel coloured pastry 'prey' in the presence of a previously trained familiar colour. They found that some birds took up to 125 trials to recruit (eat on three successive trials) this novel food into their diets, but that there was great variability between individuals. This study (Marples *et al.* 1998) also demonstrated that wild birds exhibit a greater degree of neophobia than laboratory birds (Marples & Kelly 1999). Such results reveal the importance of 'wild' studies to examine the behaviour of animals in nature.

1.3.9 Other studies

A number of small mammal and bird species have had their neophobic responses studied, in order to control vermin or reduce crop or stored food consumption (see Brigham & Sibly (1999) for references). Neophobia has been studied in relation to a number of behavioural facets; the more specialised a feeder, the greater its latency to investigate novel habitats (Greenberg 1983; Webster & Lefebvre 2000). Similarly, birds showing a greater degree of ecological plasticity are considered to be less neophobic (Greenberg 1989; Greenberg 1990a, b). Environmental experience of young moustached warblers (*Acrocephalus melanopogon*) is thought to influence their choice of feeding areas, and also their willingness to explore novel habitats (Raach & Leisler 1989).

Apart from birds and small mammals, neophobia has also been reported in slugs (Delaney & Gelperin 1986), tropical fish (Roberts & Cheney 1974), garter snakes (Czaplicki *et al.* 1975), cats (Bradshaw 1986), dogs (Vavilova & Kassil 1981), foxes (Rekila *et al.* 1999), lambs (Burritt & Provenza 1989), pigs (Dantzer & Mormede 1981), rhesus macaques (Johnson 2000), gorillas (Ogden *et al.* 1990) and humans (Birch *et al.* 1987; Pliner *et al.* 1993; Pliner & Stallberg-White 2000).

1.4 <u>Frequency-dependent selection:</u>

1.4.1 Introduction and Definitions

Tinbergen (1960), following studies on the feeding behaviour of songbirds (in particular the great tit *Parus major*), recognised that predators concentrate on common varieties of prey, and tend to overlook rarer forms even if they are obvious. He found that selection for the commonest form was strongest at intermediate densities, and weaker at high and low densities. Tinbergen (1960) assumed that birds did not adopt search images at low densities, as this was unprofitable, furthermore, he proposed that at higher densities the birds had to stop using a search image in order to maintain a sufficiently varied diet.

Apostatic selection (Clarke 1962 following Tinbergen's work), also known as reflexive selection (Moment 1962; Owen & Whiteley 1986), switching (Murdoch 1969) and matching selection (Bond 1983) is the tendency of predators to feed on a prey type when common, and ignore it when rare. Anti-apostatic selection (Greenwood 1984; Greenwood 1985), also known as unifying selection (Pielowski 1959; Pielowski 1961), oddity selection (Bond 1983) and aposematic selection (Thompson 1984) is the reverse

process, where predators feed on a prey type when rare, and ignore it when common. By their nature, these processes are described as frequency-dependent selection. The idea of frequency-dependent selection has interested researchers since at least the late 19th century (Poulton 1884), although it wasn't clearly demonstrated in wild birds until more recently (Allen & Clarke 1968). Following Allen (1988) I will use the terms 'apostatic selection' where the choice of the predator acts against the common forms, and 'anti-apostatic selection' where the choice of the predator is against the rare forms.

1.4.2 Density Effects

Allen *et al.* (1998) demonstrated that the density of the prey determines the type of frequency-dependent selection used by a predator. At low densities ($10 - 100$ baits m^{-2}) selection was apostatic, whereas at high densities ($1,000 - 10,000$ baits m^{-2}) selection was anti-apostatic. The resultant linear regression produced by the authors predicts that selection switches from being apostatic to anti-apostatic at a density of about 131 baits m^{-2} (95% confidence limits being $61 - 248$ baits m^{-2}). Other authors have found variations in these values, but may have been using different colours or sizes of prey. Raymond (1984) found that wild birds (a mixture of blackbirds, robins, song thrushes (*Turdus philomelos*), house sparrows (*Passer domesticus*), chaffinches (*Fringilla coelebs*), magpies (*Pica pica*) and woodpigeons (*Columba palumbus*)) fed anti-apostatically on baits at a density of 30 baits m^{-2}, but the patterns of the two prey differed (striped and unstriped) where Allen *et al.* (1998) had used monochrome baits. Cook and Miller (1977) found variations in the strength of apostatic selection at low to intermediate densities ($<= 7.5$ baits m^{-2}), but did not use high densities (> 500 baits m^{-2}). Willis *et al.* (1980) also identified such variations, but could detect no consistent frequency-dependent effect (bait densities were recorded comparatively, but not definitively).

1.4.3 Apostatic Selection

If the prey density is low, then the background will not contain any other prey. Under these circumstances it is assumed that predators will select the prey that is most frequently encountered, so apostatic selection is favoured. Greenwood (1984) proposed four criteria which would make apostatic selection likely: i) if there are costs related to a mixed diet, ii) if the optimal search time is different for different prey, iii) if the predator is required to sample the prey to determine its value, or iv) if the prey are Batesian mimics (i.e. palatable mimics of a toxic model). Endler (1988) suggests that apostatic selection

may be explained by an aversion of predators to rare or novel prey (see sections on neophobia above and dietary conservatism below), learning to recognise food (see point iii above), and digestion efficiency (i.e. costs related to a mixed diet - see point i above).

1.4.4 Anti-apostatic Selection

If the prey density is very high, then the background to any particular prey is composed mainly of more prey. This means that the common morph effectively becomes the background. The rare morphs thus become conspicuous on this background, and are selected by the predators (Allen *et al.* 1988). Wilson *et al.* (1990) investigated the possibility that the strength of anti-apostatic selection would increase as the prey increased their speed of movement. They conducted experiments with dense aggregations of *Calliphora* larvae on a bird table. By varying the temperature of the larvae, they were able to vary their speed of movement. Over a range of three different temperatures, and therefore speeds, they found that the strength of anti-apostatic selection by the birds (blackbirds) increased with the prey's speed of movement. These results agree with the work of Oghuchi (1981). Wilson *et al.* (1990) suggested that this effect was caused by the birds concentrating on the rare colours in the mass of fast-moving maggots to minimise the 'confusion effect'. Such an idea is reinforced by studies on predators of schooling and aggregating species. Experimental evidence has shown anti-apostatic selection to be in effect when goshawks (*Accipiter gentilis*) feed on mixed flocks (i.e. containing light and dark individuals) of pigeons (*Columba livia*) (Pielowski 1959; Pielowski 1961), sticklebacks (*Gasterosteus aculeatus*) feed on mixed aggregations (i.e. containing yellow and red individuals) of water fleas (*Daphnia magna*) (Oghuchi 1978; Oghuchi 1981) and bass (*Micropterus salmoides*) feed on mixed shoals (containing natural coloured and blue-dyed individuals) of minnows (*Hybognathus nuchalis*) (Landeau & Terborgh 1986).

Despite the studies detailed here, there is much more information to be collected before the causes of density-dependent selection are fully understood. There have yet to be many detailed studies with wild predators on wild populations, to gain observations on 'real world' effects. Such studies are essential to an ultimate understanding of density-dependent strategies in predator-prey coevolution.

1.5 Dietary Conservatism:

1.5.1 Introduction

Owen (1977) describes birds as 'conservative opportunists', whose foraging activity is heavily dependent on previous experience. Owen believed that the clutch sizes of tropical breeding birds were limited by the amount of food they could provide for their young. While appreciating that there is no shortage of invertebrate prey for passerine species, those birds need to have previous experience of the prey before they are prepared to eat them or feed them to their young. The confusing diversity of prey, in tropical forests especially, restricts the diets of the birds. Tropical invertebrate communities tend to be more diverse, but less individually numerous, than temperate ones. So temperate breeding birds have more prey available to them per 'safe species' than tropical breeding birds.

1.5.2 Original Experiment

Dietary conservatism is a term coined very recently (Marples *et al.* 1998), but relates back to the ideas of Owen (1977). The term is used to describe the slow incorporation of novel palatable prey items into the diets of predators. Marples and her colleagues were studying the responses of blackbirds and robins to novel coloured pastry. They found that having trained their birds to one colour of pastry bait, the birds were remarkably slow to incorporate a novel colour (up to 125 presentations). Dietary conservatism (DC) is not considered to be an aversive process. Unlike avoidance learning, the predator does not directly associate an unpleasant consequence with sampling the food, but is unsure if the food will be palatable or not. The distinction between neophobia and DC is not immediately clear. Perhaps it is best to consider that neophobia relates to proximity or contact with the novel food item, whereas DC relates to actually eating it (Marples & Kelly 1999).

1.5.3 A Working Definition

Having previously defined neophobia (section 1.2.1) as the avoidance of novel objects, including food, I would like to propose DC as the next step in the process of dietary incorporation. So neophobia is the initial hesitation to approach novel food, while DC is the period of time between first approach to and complete incorporation of a novel food. At the point of first approach to a novel food, by previous definition (section 1.2.1), a bird is no longer showing neophobia, as it has ceased to avoid the food. There inevitably

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follows a period of time (albeit very small in some cases) when the bird has to decide whether or not to eat this new food. This period is dietary conservatism. Such a definition does mean that DC is not interchangeable with other intuitively similar terms. Food neophobia (Roberts & Cheney 1974; Jones 1987; Pliner & Hobden 1992; Cheney & Miller 1997; Johnson 2000), feeding neophobia (Greenberg 1984; Greenberg 1990a; Dutoit *et al.* 1991; Greenberg 1992; Sunnucks 1998; Webster & Lefebvre 2000), gustatory neophobia (Ayyagari *et al.* 1991; Pelleymounter & Cullen 1993; Roozendaal & Cools 1994; Hamm *et al.* 1995) and ingestional neophobia (Franchina & Slank 1989; Franchina *et al.* 1994) all refer to the period between first encounter and first consumption of the food. These terms effectively incorporate both neophobia and DC as I have considered them, so cannot be directly compared to either.

1.5.4 *Evidence from the Neophobia Literature*

When Coppinger (1969) was considering the non-random selection of food, he cited a number of authors who had demonstrated that predators prefer familiar food (Tinbergen 1960; Beukema 1963; de Ruiter & Beukema 1963; Rabinowitch 1965; Rabinowitch 1968). Rabinowitch (1968) himself refers to a study by Neff and Meanly (1957). Neff and Meanly found that red-winged blackbirds were feeding in an area of newly seeded *Lespedeza* fields (a bush-clover forage crop), but the stomach contents of the birds contained not a single *Lespedeza* seed. Caged wild red-winged blackbirds were then tested with a mixture of rice and *Lespedeza* seed. The rice was taken, but the *Lespedeza* was refused, even when the birds were starving to death. Neff and Meanly suggested that their birds did not recognize *Lespedeza* as food. This is peculiar, as *Lespedeza* is a significant part of the diets of several other bird species (Martin *et al.* 1951), and contains no recognised toxins. This is probably the first demonstration of dietary conservatism in birds.

Rabinowitch (1965) conducted a similar experiment on induced food preferences. Six domestic chicks were raised on milo (sorghum) and eight on wheat (corn), for a period of 42 days. At this point the two groups of chicks had their diets switched, so both groups were faced with novel food. Two of the milo-trained birds, and five of the wheat-trained birds starved to death without sampling the novel food. A later experiment, also by Rabinowitch (1968) used a slightly less extreme protocol. He trained a group of herring gull (*Larus argentatus*) and ring-billed gull (*L. delawarensis*) chicks on one of three food

types: chopped worms, pink (normal) cat food, and bright green (dyed) cat food. Having been trained on these foods for five days, each bird was then given a choice either: between the training food and one of the novel foods; between the training food and the other novel food; or between the two novel foods. Of the 60 trials conducted with herring gulls, the chicks ate familiar food in 53. Of the 72 trials conducted with ring-billed gulls, the chicks ate familiar food in 69. In the tests involving only novel foods, worms were preferred over pink cat food and pink cat food was preferred over green. A number of chicks failed to take any food during these novel food trials, suggesting a lack of recognition of the material as food. Rabinowitch concluded that the chicks were basing their food preferences on familiarity.

Coppinger (1969, 1970) demonstrated neophobic reactions of birds to unfamiliar prey (see section 1.2). However, he also demonstrated DC. A number of birds originally demonstrated 'fearful' reactions to the butterflies. As the experiment continued, these birds stopped showing those reactions, but still did not attack the butterflies. He proposed that, "any original mutation which tends to make a prey species more bizarre in its natural environment may lessen the chances of that individual being immediately sampled". These 'more bizarre species' would be gaining protection both from neophobia and DC.

1.5.5 *Evidence from the Frequency-dependent Selection Literature*

Analysis of the literature on frequency-dependent selection (see section 1.3) has yielded a number of other examples of dietary conservatism prior to Marples *et al.* (1998), although they were not described as such at the time. Allen and Clarke (1968) scattered approximately 2000 green baits on a dark soil area of $40m^2$ over six days. They then tested the blackbirds feeding at that site with a mixture of 40 green and 40 brown baits over the next three days. They found that the birds favoured the familiar colour on all three days, although by the third day this bias was waning. This experiment was repeated with the colours reversed, and the same result was obtained. Raymond (1984) found that wild birds (blackbird, robin, song thrush, chaffinch, house sparrow, magpie and woodpigeon) trained to eat plain green pastry baits (over a period of 5 days), were reluctant to sample novel striped (red/green) 'morphs' of those baits when they were presented in equal numbers with the original 'morph' (over a 3 day period). Similarly, birds trained on the striped morphs, were reluctant to sample the plain green morphs. Harvey *et al.* (1975) and Allen *et al.* (1988) tested the preferences of song thrushes to the colour patterns of the snail *Cepaea*

hortensis, and found that they preferred to feed on a familiar morph. Allen *et al.* (1988) trained their birds to eat either a yellow unbanded or a yellow five-banded morph of the snail. When tested with both morphs after this training period, the birds preferred the morph they had been trained on. Their findings support the idea that polymorphism in the snail species could be maintained by apostatic selection (see section 1.3). Beissinger *et al.* (1994) found that another snail-eating bird, the snail kite (*Rostrhamus sociabilis*) was also reluctant to feed on novel snail species. They presented snail kites with an unfamiliar species of snail (*Pomacea urceus*) similar to their normal prey species (*P. doliodes*). Five of the 12 test birds chose not to attack the snails. The other seven birds captured 14 snails between them. The fate of 12 of these snails was observed. The birds dropped most of the novel snails (8 of the 12) immediately (< 10s), without any attempt to extract them from their shells. Birds that did feed on these new snails were found to handle them as efficiently as their familiar prey. This suggests that it was the unfamiliarity of the snail that was causing it to be rejected by the majority of kites. *P. urceus* is known to be eaten by the limpkin (*Aramus guarauna*) amongst other birds, so was presumably not noxious or distasteful to the kites (Beissinger *et al.* 1994).

1.5.6   *Separating Choice and Fear*

The birds studied by Marples *et al.* (1998) had plenty of time to become familiar with the novel colour of the pastry baits. It would not be fair to say that they were 'afraid' of them, indeed they regularly stood close to or actually on them as they fed on the familiar baits. It is possible that like the experiments by Neff and Meanly (1957) and Rabinowitch (1961) the birds had not recognised the novel baits as food.

With the benefit of hindsight it is possible to reinterpret the genetic work of Marples and Brakefield (1995) as demonstrating DC (Marples & Kelly 1999). Perhaps the trait that was being selected for was in fact DC rather than neophobia. At the F3 generation five out of six 'FAST' birds were prepared to eat novel seven-spot ladybirds (*Coccinella septempunctata*), while only one of the six 'SLOW' birds was prepared to do so (Marples & Brakefield 1995). Comparing the consumption of the 'FAST' and 'SLOW' birds as populations over the five days of the trial gives an even more dramatic difference. The 'FAST' birds consumed 19 individuals, whereas the 'SLOW' birds consumed only one. At the same time, these two generations were shown to have no significant difference in their

neophobic responses to novel objects. This argues strongly for the treatment of neophobia and DC as different processes (Marples & Kelly 1999).

If we are to accept that neophobia relates to contact with novel food, and DC relates to actually eating it, it may be profitable to re-examine the work of a number of authors (e.g. Schuler & Hesse 1985; Roper & Cook 1989), who found that birds which were reluctant to eat a novel food, were not necessarily reluctant to peck at it.

Dietary conservatism has been poorly studied since its elucidation, yet it may well hold some answers to the evolution of aposematic or polymorphic animals (see chapter 6).

1.6 Aposematism:

1.6.1 Original Observations

Henry Walter Bates (1862) was one of the first people to contemplate that conspicuous colours of animals might be a form of protection. Although his work is more frequently remembered for his theory of mimicry, the idea of conspicuous and defended animals was a central part of this. Bates suggested unpalatability as the defence of the ithomiid butterflies he was studying, since they had no visible physical defences (spines, stings or biting mouthparts). The butterflies also produced an odour that he found mildly unpleasant, and he noted that his collections of ithomiids seemed unusually resistant to mite attack. Haase (1892) recorded that a group of supposedly unpalatable butterflies (the papilionids) ate from poisonous plants in their larval stage, although the nature of the toxins these plants provide was not elucidated until the latter half of the twentieth century (e.g. Reichstein *et al.* 1968; Brower 1969; Rothschild 1985).

Bates was not alone in his musings on the subject. Wallace (1867) reasoned that distastefulness alone would not protect a larva. It would need some sort of overt signal to convey its unpalatability to a potential predator. Guilford (1990) considers that it is from this work that the theory of aposematism has developed. Soon after Wallace's paper Darwin (1871) suggested that it would be highly advantageous for a noxious larva to be instantaneously recognised by a predator, and to this end "the most gaudy of colours would be serviceable". These colours would have evolved from the most memorable individuals that survived their predators' attention.

Edward Poulton coined the term aposematic coloration (1890). He defined it as "an appearance which warns off enemies because it denotes something unpleasant or

dangerous; or which directs the attention of an enemy to some specially defended, or merely non-vital part; or which warns off other individuals of the same species". It is the first part of this definition that is generally used to represent aposematism nowadays.

1.6.2   A working definition

Although Bates (1862) and Wallace (1867) were considering 'distastefulness', it is likely that toxins protected the butterflies they were observing too. By substituting the general term 'unprofitable' into Poulton's definition (above), we can cover the spectrum of misfortune to the predator, from inconvenience to fatality. Predators do not rely solely on visual cues to hunt by, so it seems natural enough that aposematic animals should advertise in other sensory modalities. Aposematic coloration no longer appears to be a sufficient term to describe the variety of advertising signals these animals use. Aposematic animals may employ warning sounds (Dunning & Kruger 1995; Lourenco & Cloudsley-Thompson 1995; Marshall *et al.* 1995; Kinney *et al.* 1998; Kirchner & Roschard 1999), odours (Eisner & Grant 1981; Rothschild & Moore 1987; Peck 2000), bioluminescence (Underwood *et al.* 1997; de Cock & Matthysen 1999) and even movements (Henrikson & Stenson 1993; Hatle & Faragher 1998). With this in mind, I would like to propose a more general definition of an aposematic animal, *viz.* 'an unprofitable species which advertises its presence with conspicuous signals'. Here the term 'unprofitable' may include animals that are difficult to catch (Baker & Parker 1979), as well as those that are toxic or otherwise harmful (Guilford 1990).

1.6.3   Multimodal Defences

As an aposematic species, it would seem prudent to advertise your unprofitability as boldly as possible. To this end, a number of species appear to use a combination of warning signals in different sensory modalities. Such animals are referred to as multimodal signallers (Rowe 1999a; Rowe & Guilford 1999a). Examples of such animals include ladybirds (Marples *et al.* 1994), soldier beetles (Pearson 1989), froghoppers (Homoptera: Cercopidae) (Peck 2000), arctiid moths (Dunning & Kruger 1996) and lubber grasshoppers (*Romalea guttata*) (Hatle & Faragher 1998). Novel multimodal signals have been demonstrated to be more effective than individual novel signals (Rowe & Guilford 1996), and even to interact synergistically (Marples & Roper 1996). Pearson (1989) discusses the adaptive significances of these defences with particular reference to tiger beetles (Cicindelidae). He considers that various defences may have arisen to counter different

foraging phases of a single predator, to counter different predators, or even from different life cycle stages. It is equally possible that multiple defences have been developed to defend against a single predator type, as multicomponency has been shown to increase the detectability, discriminability and memorability of signals by predators (Rowe 1999b).

1.6.4 Separating Multicomponent Signals

Marples *et al.* (1994) attempted to separate the components of the multimodal signal of the seven-spot ladybird (colour pattern – seven black spots on a red background; taste – reflex blood from ladybirds; smell – odours from hidden, crushed ladybirds), using laboratory-bred Japanese quail as predators. Like Wilcoxen *et al.* (1971) and Gillette *et al.* (1980) they found that colour pattern was the most effective single cue to deter predation, and smell the least effective. Both smell alone, and smell with taste were only very mild deterrents. Although the initial effect of colour pattern alone was strong, this effect decreased with time. Similarly, when colour pattern was added to a smell treatment, the initial increased rejection rate waned as quickly, if not more so. Later work (e.g. Marples & Roper 1996) has shown that although novel colour and novel odour in combination may be highly aversive, they are ultimately insufficient as a defence. When colour pattern was paired with taste and smell, the quail initially rejected these treatments almost as much as whole ladybirds, even though there was considered to be no toxic effect to the treatment (Marples *et al.* 1994). However, even here, it looked like this rejection rate was decreasing. It appears that in order to maintain rejection of the prey animal, the unprofitable aspect (in this case the toxicity) of that prey must be maintained. This leads us on into the area of learned avoidance, which is discussed in section 1.6.

1.6.5 Intended Recipients of the Message

The vast majority of work has been conducted on vertebrate predators of aposematic insects, in particular the relation between insects and birds. Nonetheless, there are a few examples of invertebrate predators being sensitive to these warning signals too (Bowdish & Bultman 1993; Kauppinen 2001). Such examples may prove to be instructive in our understanding of the evolution of aposematism, as it is likely that invertebrate predators co-evolved with their invertebrate prey for about 200 million years before vertebrates evolved.

1.7 Learning and Memory Theory:

1.7.1 Learned Aversions

Foree and Lolordo (1973) found that if pigeons were trained to press a treadle in the presence of a combined visual and auditory stimulus (i.e. a light and a tone), the relative importance of the visual and auditory cues depended on the nature of the reinforcement. Pigeons that were responding to food would not respond to the tone alone, but would respond to the light alone. On the other hand, pigeons that were trained to avoid electric shocks would respond to the tone alone, but not to the light alone. It appears therefore, that the cues to which an animal normally attends while feeding are those that are effective in learned aversions to the physiological consequences of feeding. Such associations are described by stimulus relevance (Capretta 1961) and 'belongingness' (Garcia & Koelling 1966).

Birds can readily learn aversions to the sight of food (Brower 1969). Wilcoxon *et al.* (1971) found that quail learn poisoned-based aversions more rapidly to the colour than the taste of drinking water. Moore and Capretta (1968) obtained similar results with chickens. Studies with wild and wild-caught birds have found this same effect of colour (Mason & Reidinger 1982; Mason & Reidinger 1983a, b; Martin & Lett 1985; Grieg-Smith 1987; Grieg-Smith & Rowney 1987). By facilitating avoidance learning, and increasing the memorability of prey (Speed 2000), warning colours increase the likelihood of individual survival (Gittleman *et al.* 1980; Roper & Wistow 1986). Additional cues (e.g. odours or flavours) have been found to further facilitate aversion learning (Avery & Nelms 1990; Avery & Mason 1997; Nelms & Avery 1997). So it appears that multimodal stimuli aid aversion learning. Perhaps then, one might predict that aposematic animals ought to use multimodal signals (see section 1.5).

An important aspect of aversion learning is the toxicity of the food item being consumed. Some early studies (Mühlmann 1934) discovered that bad taste and novel colour were insufficient deterrents to prevent chicks from eating mealworms. The importance of toxicity has been reiterated in later studies (Rogers 1978; Marples *et al.* 1994). Numerous attempts to defend fruit from avian predation have failed owing to the lack of toxicity of the treatment (Avery *et al.* 1993; Curtis *et al.* 1994; Cummings *et al.* 1995). Clearly an aposematic insect must ultimately develop toxicity rather than unpalatability to guarantee survival.

1.7.2   Social learning

Area copying

Also known as local enhancement (Thorpe 1956; Galef 1988), area copying occurs when an individual directs its behaviour towards the place where others are currently active (Krebs & Davies 1997). Area copying can optimise foraging in social species. For instance, in red-winged blackbirds, flocks that contained experienced individuals induced naïve birds to forage first in places with safe food but it failed to teach them to avoid places with unpleasant food (Avery 1994). Area copying can increase foraging gains, whether in terms of foraging rate or reduced risk of starvation (Caraco & Giraldeau 1981; Hake & Ekman 1988). For instance, area copying allows great tits foraging in flocks of four to find food more quickly than in flocks of two or when foraging alone (Krebs *et al.* 1972). Area copying has been modelled as an information-sharing system (Ranta *et al.* 1993; Ruxton *et al.* 1995) or as a scrounger-producer system (Barnard & Sibley 1981; Caraco & Giraldeau 1991; Vickery *et al.* 1991). Comparative model simulations have suggested that the producer-scrounger system leads to higher average feeding rates (Beauchamp & Giraldeau 1996). Recent studies with spice finches (*Lonchura punctulata*) (Mottley & Giraldeau 2000; Coolen *et al.* 2001) have shown that groups of foragers converge on predicted producer-scrounger equilibria.

Area copying appears common in social passerine species, and has been demonstrated in great tits (Krebs *et al.* 1972), house sparrows (Barnard & Sibley 1981), greenfinches (*Carduelis chloris*) (Hake & Ekman 1988), siskins (*Carduelis spinus*) (Senar & Metcalfe 1988), canaries (Cadieu *et al.* 1995a) and zebra finches (Coleman & Mellgren 1994). Domestic chicks (Tolman 1964; Jones 1983) and quail (Turro-Vincent *et al.* 1995) also show this behaviour.

Object copying

Also known as stimulus enhancement (Galef 1988) or 'releaser-induced recognition' (Suboski 1990), object copying is similar to area copying in that it directs behaviour. However, the behaviour is directed at an object that matches the type attended to by others, rather than a place (Krebs & Davies 1997). Object copying has been related to enemy recognition (Curio 1988) as well as feeding. Red-winged blackbirds acquire food aversions and preferences socially (review in Mason 1988), and it appears that house sparrows are capable of this too (Fryday & Grieg-Smith 1994). Feral pigeons avoid seed types chosen by flock members (Inman *et al.* 1988), while woodpigeons (Murton 1971) and

greenfinches (Klopfer 1961) prefer food chosen by members of their flock. Greenfinches have also been shown to incorporate new food types by such learning (Pettersson 1959).

Blackbirds display area copying (pers. obs.), but do not demonstrate object copying (Marples *et al.* 1998). A male blackbird of a pair that had witnessed the female bird feeding on the 'novel' colour on several occasions did not sample the food as a result. In fact, it was likely that this male bird only sampled the novel food as the result of an injury, when the experimental presentations became a more important addition to its diet.

Behaviour copying

Sometimes known as imitation (Galef 1988; Heyes 1993), behaviour copying occurs when a topographically novel behaviour pattern is acquired by one individual seeing another individual use it (Krebs & Davies 1997). Such behaviour appears to be limited to vocal learning in birds (Dawson & Foss 1965; Galef *et al.* 1986), so will not be discussed further here.

1.7.3 Memory and forgetting

Bouton (1994) argues that the theories of learning and memory should be considered as a single combined theory. Learning, remembering, and forgetting all occur within the same biological context, and so their adaptive functions are interrelated. It does appear that the characters of aposematic prey that accelerate aversion learning may also be the characters that decelerate forgetting processes (Speed 2000). When a predator learns about an unpleasant aposematic prey it forms a long-term memory of the warning signal, and of the prey's unprofitability. Speed (2000) considers that aposematic prey may be memorable because they are novel, conspicuous, discriminable and signal multimodally (see section 1.5). Encounters without contact may serve to prolong the predator's memory of an aposematic prey. A predator may jog its memory if it encounters the same aposematic form and avoids it. This memory jogging could serve to stop forgetting temporarily, reverse any previous effects of forgetting, and slow down any future forgetting. So such memory jogging could enhance protection of the aposeme. Such benefits would be particularly important for emerging, and therefore rare, aposematic forms (Speed 2000).

There is evidence to suggest that aversion learning of severe punishments can last longer (Kraemer & Roberts 1984) than that of milder punishments (Alcock 1970). So the nature of the encounter a predator has with an aposematic prey should determine the initial

strength of the memory the predator has (Speed 2000). The appearance of the aposeme, and any future encounters with it, would then determine the rate at which the predator forgets about it. Indeed Servedio (2000) suggests that it may be extremely difficult for warning coloration to evolve in mildly toxic prey.

This is a simplified scenario. There are additional complexities to both memory and forgetting if we deal with mimicry systems (Turner & Speed 1996; Speed & Turner 1999). My work is not involved with the investigation of these established systems, so I will not examine these complexities here.

1.8 The importance of passerines:

Previous laboratory studies have used domestic chicks or quail as experimental animals. These animals are very convenient species for the researcher as they are cheap and widely available. However, they are not necessarily good predictive models for all birds. Both species are from the family *Galliformes*. Birds of this family are known to have intermediate sized olfactory lobes (Cobb 1960a), the part of the brain associated with odour perception (see section 1.1.2). However, most bird species that would be foraging on insects and seeds belong to the family *Passeriformes*. Birds in this family have much smaller olfactory lobes, and have never been studied with respect to the effects of odour on their food choice, excepting aversion-learning studies (Avery & Nelms 1990; Nelms & Avery 1997). Experiments testing the responses of bird species from this family are clearly needed before hypotheses can be drawn about the behaviour of the majority of seed and insect-eating wild birds.

Chicks are precocial (feathered and mobile on hatching), and therefore differ fundamentally from a large number of bird species that are altricial (blind and naked young). Altricial chicks are fed in the nest for a number of weeks before fledging. This 'priming' of the young by their parents has been shown to influence their future food choices (Cadieu *et al.* 1995b; Avery 1996). Passerines produce altricial young that have no opportunity to forage until fledging. Indeed, a number of species do not forage independently until some time after this (Cramp 1998). With this in mind it may be difficult to consider any altricial fledgling as truly naïve, since it has already acquired some feeding experience from the food choices made by its parents. At fledging time (or independence) there may be dramatic differences in the way juvenile passerines forage

initially, compared to galliforms. These differences may increase or decrease with experience, but could result in very different behaviours of adult birds.

Studies on domestic chicks have shown that the hemispheres of the brain undergo dramatic changes in their cognitive responsibilities during the first two weeks of life (Andrew 1988; Vallortigara & Andrew 1994; Regolin & Vallortigara 1996). This is precisely the period of time that chicks are used for the majority of studies. It would be reassuring to know that these changes did not confound experimental results. Furthermore, most chicks used in experiments are provided by the poultry industry. Males are considered 'waste' products of this industry, as they don't lay eggs. Consequently most chicks used in experiments are male. Clearly by using a more even sex ratio it should be possible to draw more robust conclusions.

Working with domestic chicks inevitably means working in the laboratory. This is a convenient environment for the researcher, but does it replicate the wild environment sufficiently for the test animal? This project will investigate the responses of both laboratory-held and wild adult passerine species to odour and colour signals in food in more detail than has previously been attempted. Results from these experiments will be directly relevant to the development of deterrent treatments for crops as well as demonstrating the generality of the results found using quail and domestic chicks.

1.9 <u>Experimental Plan:</u>

The bright colours and characteristic odours of many insects may act as warning signals of their toxicity to bird predators (see section 1.5). Communication systems between predators and prey, such as these warning signals, provide some of the best examples of co-evolution and have therefore been central to studies of the mechanisms of evolution (Guilford 1988; Guilford 1990; Guilford & Cuthill 1991; Guilford & Dawkins 1991; Rowe & Guilford 1999b). At the same time such systems are of great practical interest, since a firm understanding of how birds make foraging decisions is vital both to those needing to defend crops from avian damage, and those seeking to conserve bird species. This project aims to provide fundamental information relevant to both applied and theoretical aspects of avian biology.

Most work on warning signals between prey and predator species has concentrated on the effects of colour patterns alone, but recent work (Marples & Roper 1996; Rowe &

Guilford 1996) has shown that the odours present in the signalling insects may be at least as important as the colour. The effects of odour on the learning processes of birds are very poorly understood. Work using a combination of cues has shown that food avoidance may be enhanced by the addition of a novel odour (Marples & Roper 1996; Rowe & Guilford 1996; Marples & Roper 1997), and memory may be stronger when flavour is present too (Marples *et al.* 1994). It has also been shown that domestic chicks use some odours in preference to colour for learned avoidance (Roper & Marples 1997b). Most previous work has used artificial odours, so this project followed procedures developed during these earlier experiments, but used odours taken from natural signalling systems. The effects of independent and combined novel colour and novel odour cues on learning and memory was investigated in more detail than in previous studies.

Studies carried out in the laboratory can only generate hypotheses about the behaviour of wild birds. Experiments under natural conditions are vital if we are to understand the actual responses of birds to novel, odorous foods. This study addressed this need using two different approaches. Experiments on populations of individually marked wild birds were carried out which complemented the laboratory studies and demonstrated the responses of these birds to colour and odour presented in a controlled dosage on artificial food items. In addition, a field trial was carried out to assess the practicality of odour and colour treatments as bird aversants.

Birds are attracted to grain storage silos and can both soil and remove significant quantities of the crop (Wildey *et al.* 1999). Birds are 'controlled' as a measure to reduce this damage, but it would be more cost effective, not to mention more acceptable with regards to animal welfare, to repel them from the food if this could be done without damaging the grain. Similarly, crops planted on the surface of the soil, such as grain, may suffer significant predation before or during germination (Pascual *et al.* 1999). Field trials conducted as part of this study demonstrated whether colour and odour can be used as an effective protection of such seed during storage.

Finally, the project investigated the response of birds to colour and odour signals using three interdependent approaches; laboratory studies of zebra finches and domestic chicks, experiments on individually identified birds in the wild, and field trials of colour and odour as avian aversants to social species. This combination of studies improved understanding of the effects of colour and odour perception. This knowledge will have practical applications and will contribute to the elucidation of predator-prey co-evolution.

Chapter 2

The responses of laboratory-held adult passerines to novel foods with combinations of novel colour and novel odour

2.1 <u>Introduction:</u>

The warning colours of aposematic animals advertise their unprofitability to predators (Cott 1940; Edmunds 1974) (section 1.5). These colours facilitate avoidance learning (Bullard *et al.* 1983; Mason & Reidinger 1983b; Martin & Lett 1985; Grieg-Smith & Rowney 1987; Roper & Marples 1997a) and increase memorability (Speed 2000), and therefore increase the likelihood of individual survival (Gittleman *et al.* 1980; Roper & Wistow 1986). Chicks have been demonstrated to show a synergistic reaction to a bimodal signal consisting of novel colour and novel odour cues (Marples & Roper 1996). This demonstrates clearly that chicks notice the presence of a novel odour, even if they do not react to it as an independent cue. Having previously expressed reservations about the use of chicks as general models (section 1.7), it is important to examine these reservations. Galliforms are known to have relatively larger olfactory bulbs than finches (Bang & Cobb 1968), but this doesn't necessarily mean that finches have a poorer sense of smell (Clark *et al.* 1993). This experiment aimed to test whether the zebra finch (a commonly held passerine species) is capable of detecting odours, and whether it displays a synergistic response to multimodal cues in the same way that chicks do.

2.2 <u>Materials and Methods:</u>

The zebra finches were housed as same sex pairs in wooden cages (30cm x 35cm x 60cm) with a 1cm wide vertical slot in the centre of the back wall. This slot allowed the insertion of a plastic divider to separate the cage into two equal areas during the trials. The birds were maintained in a 12h/12h light/dark regime.

Training food was a high nutrient food (CéDé® Egg Food for Canaries, CéDé® Vogelvoeders B.V., StCeciliastraat 2, 5038 HA Tilburg, The Netherlands), which was made up into a rough paste with water (approx. 50/50 mixture). The birds were familiarised with the training food as a pair over a period of a week. Food was presented daily, in a white plastic dish (6cm diameter, 2.5cm deep) and left for the birds to investigate. The training food was normally preferred to the birds' usual food (foreign finch seed – PetStop Superstores). At testing time, the subjects were tested individually in divided cages. In order to reduce the stress of isolated birds (Sillén-Tullberg 1985), a number of boxes were tested simultaneously. This allowed vocal communication between the individuals under test, but no observation of the choices made by those individuals. The birds were deprived

of food for 90 minutes, and then tested with one of four treatments: control (C – the training food), novel odour (NO – the training food in the presence of pyrazine), novel colour (NC – the training food made up with red dye) and doubly novel (NCO - the training food made up with red dye, and in the presence of pyrazine). The pyrazine was a 0.0003% solution of 2-methoxy-3-isopropylpyrazine (Pyrazine Specialties Inc. P.O. Box 6933, Atlanta, Georgia 30315, USA), as this has been previously identified as being similar in intensity to undiluted almond essence (Marples & Roper 1996). The red dye was a 25% solution of O'Brien's Christmas Red (4R) (O'Brien's Ingredients, O'Brien House, Ballymoss Road, Sandyford Industrial Estate, Dublin 18, Ireland), as this had been found to be similar in intensity to other useful food dye colours (chapter 3). All feeding cups contained a band of filter paper on their inner rim, which was soaked with water (when no odour was required) or the solution of pyrazine.

For the test, birds were presented initially with the control food, then the test treatment, and then the control food again. This protocol allowed the birds to demonstrate their habituation to the test conditions. If a bird refused to eat the initial presentation of the C food within 10 seconds, it was considered to be 'uncomfortable' in the test environment, and was not given the subsequent presentations. Birds display fear by adopting frozen postures known as tonic immobility [Ratner, 1967 #296]. As birds usually responded immediately to the presentation of the C food, those that did not respond were considered to be 'uncomfortable'. Latencies to contact (peck at) and to incorporate (eat on three successive presentations within 10 seconds) the treatments were recorded. Once a treatment had been sampled, it was removed, and replaced with C food. This acted as a second test on the welfare of the birds. If birds failed to eat the C presentation on this second presentation within 10 seconds, then their previous test presentation was discarded. Birds were allowed up to a maximum of one hour with their test foods. This meant that birds were never without food for more than two and a half hours (following EC directive 86/609/EC). As birds were not guaranteed to eat a particular treatment on their first presentation, the latencies from consecutive trials were added together to derive a final total latency score. When birds had eaten a given treatment on three consecutive presentations, in under 10 seconds (i.e. it was eaten as rapidly as control food), they were considered to have incorporated that food. Incorporation was recorded retrospectively, as the first of the three consecutive presentations that the bird had eaten the food. A number of birds were observed to sample food on single occasions, and subsequently refuse that same

food. By ensuring that the food was eaten on three successive presentations, there was no confusion as to the acceptance of the food by the bird. Latencies were recorded as the number of seconds and the number of presentations before contact and incorporation, in order to allow later comparison.

In order to reduce experimental numbers, in keeping with general welfare considerations (Russell & Burch 1959), all birds were tested on two different treatments. By pairing the C and NCO and the NO and NC treatments, it was possible to maintain the novelty of the colour and odour cues prior to testing. To allow for any possible order effects of these paired treatments, one half of each group received one treatment first and the other second, while the other half received them in the reverse order. Each paired treatment group was originally assigned 12 birds. This allowed six birds in each half to test for order effects. Unfortunately, owing to natural mortality and unknown infectious agents, the final group sizes were not so uniform. The actual number of birds presented with each treatment were as follows: C = 9, NO = 20, NC = 16, NCO = 9.

A further test was conducted on a number of longer-lived individuals. Eight months after originally incorporating the NC treatment, these birds were tested with it again. The gap of eight months was considered sufficient to represent the return of a natural season. The memory of birds over this period of time could be important in determining their reactions to seasonally abundant prey species.

2.3 **Data Analysis:**

Statistical tests were performed on SPSS© v10.0.5 (SPSS Inc. 1989 - 1999). As the latencies were not normally distributed (Shapiro-Wilk $p < 0.05$), the data were analysed non-parametrically.

2.4 **Results:**

There were no order effects for any of the treatments (Mann-Whitney U tests: C: U = 9.0, n = 3, 6, p = 1.00, NO: U = 28.0, n = 6, 14, p = 0.15, NC: U = 19.0, n = 6, 9, p = 0.35, NCO: U = 4.0, n = 3, 5, p = 0.29), so the data (see appendix 1) were combined for analysis. Some of the birds had not incorporated the food by the end of the experimental period, so minimum possible values (MPVs), were calculated for them. MPVs were

calculated by taking the cumulative total latencies of birds that had not incorporated, and adding either one second or one presentation to them. This gave a value of the shortest possible time any bird could have taken to incorporate a treatment. These values are almost certainly conservative, as none of the birds were sampling the food regularly prior to discontinuing the trials. Mean latencies to contact and incorporate the four treatments are shown in figures 2.1 and 2.2, either as the number of presentations or the time taken (in seconds) until the event. Analysis of the time data showed a difference in the latencies to incorporate the four treatments (Kruskall-Wallis test adjusted for ties: $\chi^2 = 38.3$, df = 3, $p < 0.001$). Subsequent analysis of the individual treatments are summarised in table 2.1. The only comparison that failed to produce a significant difference was between the C and NO treatments. This shows that there was no

	Control	Novel Odour	Novel Colour
Novel Odour	0.889		
Novel Colour	< 0.001	< 0.001	
Doubly Novel	< 0.001	< 0.001	< 0.001

Table 2.1. Comparison of incorporation latencies for the four treatment groups using Mann-Whitney U-tests. Values quoted are 2-tailed p values adjusted for tied ranks.

main effect of odour. As all treatments containing colour were significantly different from those without colour (Table 2.1), this showed a main effect of colour. There was also a significant difference between the NC and NCO groups (Table 2.1). This demonstrated an interaction between novel colour and novel odour. The interaction between novel colour and novel odour can be described as synergistic, as it was greater than the combined effects of these two independent cues. The mean incorporation time for NO was 4 ± 0.6s (n = 20), and for NC was $13,052 \pm 2290$s (n = 15). This gives an additive incorporation time of $13,056 \pm 2291$s, but the actual mean incorporation time for NCO was $43,555 \pm 5711$s (n = 9). Some birds were taking over 10 weeks of continuous testing before incorporating the NCO treatment, as only one trial was conducted on any individual per day.

The results of the long-term memory test are shown in figure 2.3. The four birds tested showed a greatly reduced (and almost negligible) latency to incorporate the red food on the second occasion. This suggests that the birds were treating the NC food as familiar, despite not having seen it for eight months.
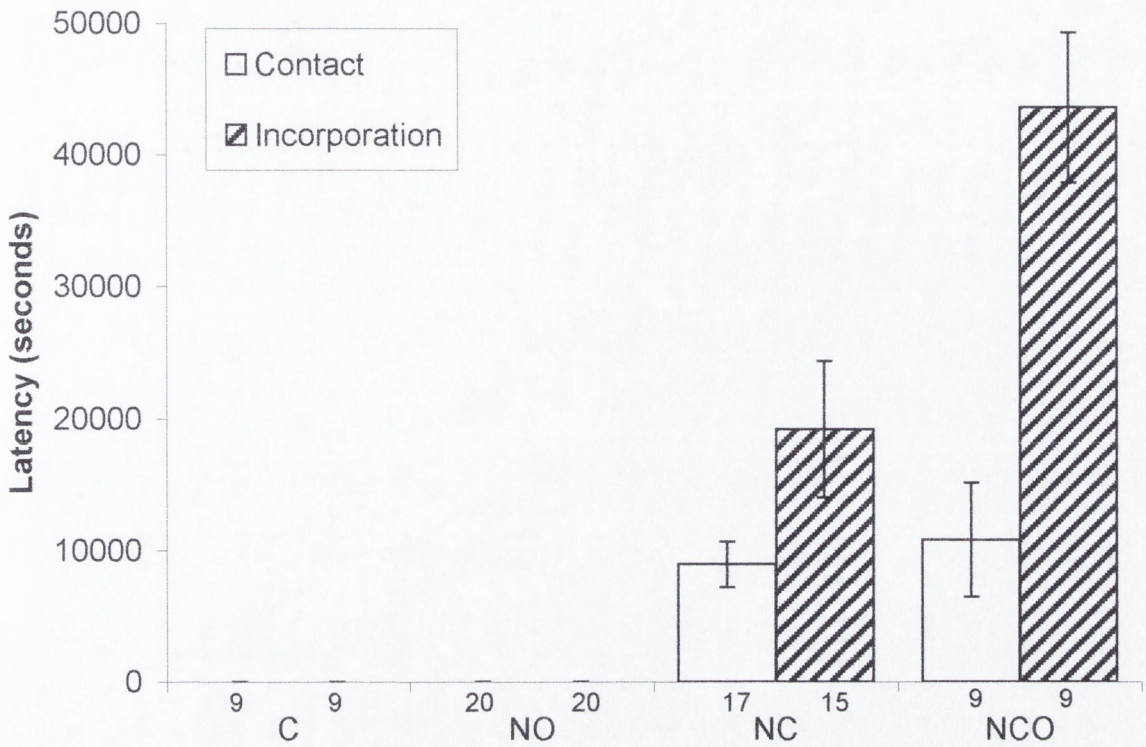
Figure 2.1. Comparative latencies (s) of zebra finches to contact and incorporate three novel treatments of food. Control (C) and Novel Odour (NO) values were insignificant in comparison to the Novel Colour (NC) and Novel Colour and Odour (NCO) treatments so appear only as titles on the x-axis. Standard errors of the mean are shown where they are significantly greater than zero, and sample sizes are shown below the respective treatments.

Figure 2.2. Comparative latencies (presentations) of zebra finches to contact and incorporate three novel treatments of food. Standard errors of the mean are shown where they are significantly greater than zero, and sample sizes are shown below the respective treatments.

Figure 2.3. Comparative latencies (s) of four zebra finches to incorporate novel coloured (red) food on two occasions separated by a period of eight months.

A.

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1                                                          GTCA
5   TCTCCGTCACTCCCTTTTTCTCTACACAGATCTCTCATTCACTCTCTCGTACACA
    Met Gln Ser Val Leu Ser Leu Ser Phe Ser Gln Ala Ser Leu   14
60  ATG CAA TCG GTT CTT TCT CTT TCC TTC TCA CAA GCA TCG CTT
    Pro Leu Ala Asn Arg Thr Leu Cys Ser Ser Asn Ala Ala Pro   28
102 CCT TTA GCG AAT CGT ACG CTT TGT TCA TCC AAC GCA GCT CCT
    Ser Thr Pro Arg Asn Leu Arg Phe Cys Gly Leu Arg Arg Glu   42
144 TCT ACG CCG AGA AAT CTC CGG TTC TGT GGA CTC CGG CGA GAA
    Ala Phe Cys Phe Ser Pro Ser Lys Gln Leu Thr Ser Cys Arg   56
186 GCG TTT TGC TTC TCT CCG TCG AAG CAA TTG ACC TCG TGC CGT
    Phe His Ile Gln Ser Arg Arg Ile Glu Val Ser Ala Ala Ala   70
228 TTC CAT ATT CAG AGT AGG AGA ATC GAA GTC TCC GCC GCT GCT
    Ser Ser Ser Ala Gly Asn Gly Ala Pro Ser Lys Ser Phe Asp   84
270 TCT TCT TCC GCT GGA AAT GGA GCT CCA TCG AAA TCA TTC GAT
    Tyr Asp Leu Ile Ile Ile Gly Ala Gly Val Gly Gly His Gly   98
312 TAT GAT TTG ATC ATT ATC GGA GCT GGA GTT GGT GGC CAT GGA
    Ala Ala Leu His Ala Val Glu Lys Gly Leu Lys Thr Ala Ile   112
354 GCT GCA TTG CAC GCC GTC GAG AAG GGA CTC AAA ACT GCT ATC
    Ile Glu Gly Asp Val Val Gly Gly Thr Cys Val Asn Arg Gly   126
396 ATT GAA GGA GAT GTT GTT GGA GGT ACT TGC GTT AAC AGA GGC
    Cys Val Pro Ser Lys Ala Leu Leu Ala Val Ser Gly Arg Met   140
438 TGT GTG CCT TCC AAA GCT CTA CTT GCT GTT AGT GGT AGG ATG
    Arg Glu Leu Gln Asn Glu His His Met Lys Ala Phe Gly Leu   154
480 AGG GAA CTC CAG AAC GAA CAT CAC ATG AAG GCT TTT GGT TTG
    Gln Val Ser Ala Ala Gly Tyr Asp Arg Gln Gly Val Ala Asp   168
522 CAG GTT TCA GCT GCT GGT TAT GAC CGC CAA GGT GTG GCT GAC
    His Ala Ser Asn Leu Ala Thr Lys Ile Arg Asn Asn Leu Thr   182
564 CAC GCA AGT AAC CTG GCT ACC AAA ATT AGG AAT AAT CTC ACC
    Asn Ser Met Lys Ala Leu Gly Val Asp Ile Leu Thr Gly Phe   196
606 AAT TCT ATG AAG GCA CTT GGT GTT GAC ATA TTG ACA GGG TTT
    Gly Ala Val Leu Gly Pro Gln Lys Val Lys Tyr Gly Asp Asn   210
648 GGC GCT GTT CTG GGC CCA CAA AAG GTT AAA TAT GGT GAC AAT
    Ile Ile Thr Gly Lys Asp Ile Ile Ile Ala Thr Gly Ser Val   224
690 ATT ATC ACC GGA AAA GAT ATA ATC ATC GCA ACT GGA TCT GTA
    Pro Phe Val Pro Lys Gly Ile Glu Val Asp Gly Lys Thr Val   238
732 CCG TTC GTC CCG AAA GGA ATT GAA GTT GAT GGA AAG ACT GTT
    Ile Thr Ser Asp His Ala Leu Lys Leu Glu Ser Val Pro Asp   252
774 ATC ACA AGT GAT CAT GCA TTG AAA TTG GAG TCC GTT CCT GAC
    Trp Ile Ala Ile Val Gly Ser Gly Tyr Ile Gly Leu Glu Phe   266
816 TGG ATT GCG ATA GTA GGA AGT GGT TAT ATC GGT CTT GAG TTC
    Ser Asp Val Tyr Thr Ala Leu Gly Ser Glu Val Thr Phe Ile   280
858 AGT GAT GTT TAC ACG GCC CTT GGA AGT GAG GTA ACT TTT ATT
    Glu Ala Leu Asp Gln Leu Met Pro Gly Phe Asp Pro Glu Ile   294
900 GAG GCA CTG GAT CAA CTA ATG CCT GGA TTT GAT CCT GAG ATC
    Ser Lys Leu Ala Gln Arg Val Leu Ile Asn Thr Arg Lys Ile   308
942 AGT AAG CTG GCT CAA AGG GTT CTA ATA AAT ACA AGA AAA ATT
    Asp Tyr His Thr Gly Val Phe Ala Ser Lys Ile Thr Pro Ala   322
984 GAC TAC CAT ACT GGA GTA TTT GCA AGC AAA ATC ACT CCA GCA
    Lys Asp Gly Lys Pro Val Leu Ile Glu Leu Ile Asp Ala Lys   336
1026 AAG GAT GGG AAA CCA GTG CTG ATT GAA CTA ATT GAT GCC AAA
    Thr Lys Glu Pro Lys Asp Thr Leu Glu Val Asp Ala Ala Leu   350
1068 ACC AAG GAA CCC AAG GAT ACT TTG GAG GTT GAC GCT GCT CTA
    Ile Ala Thr Gly Arg Ala Pro Phe Thr Asn Gly Leu Gly Leu   364
1110 ATT GCT ACT GGA AGA GCT CCA TTC ACC AAT GGT CTT GGC CTG
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Figure 2.3 A. Nucleotide and translated sequence of dld.ara1 cDNA. Start and stop codons are highlighted in bold (**cont'd**).

2.5 **Discussion:**

Zebra finches react in the same way towards novel food as chicks do (Marples & Roper 1996). This result is interesting for a number of reasons. Importantly, it indicates that chicks are a useful model for zebra finches, under laboratory conditions. With obvious differences between altricial (e.g. passerines) and precocial (e.g. galliforms) species, this might not have been predicted (see section 1.7). Equally importantly, it indicates that zebra finches possess a sense of smell, and use this sense to determine the acceptability of novel foods. Clark *et al.* (1993) identified that passerines have similar odour thresholds to galliformes, despite having relatively smaller olfactory bulbs (Bang & Cobb 1968) (see section 1.1.2). Clark *et al.* (1993) also highlighted an important difference between an individual's capacity and its tendency to attend to an odour cue. That is, just because a bird has the ability to detect an odour, it doesn't mean that it will attend to the cue when it encounters it. Here is a clear example of a passerine attending to such a cue.

Zebra finches demonstrated a synergistic effect of novel colour and novel odour in the same way that chicks have been shown to (Marples & Roper 1996). Here we have further evidence of the efficacy of multimodal defences incorporating colour and odour (see section 1.2.5).

The literal definition of neophobia is 'fear of the new' (Barnett 1958), but this definition is not precise enough for our experimental conditions. Brigham and Sibly (1999) define neophobia as "the initial avoidance of novel objects in an otherwise familiar environment", where "objects" may include food. The experimental feeding environment was unmodified once birds began to be tested, so the presence of test food would only have been truly novel on the first presentation. The unfamiliarity of novel objects must decrease with increased exposure to them, and hence neophobia decreases with experience (see section 1.2). It is reasonable to assume that some birds exhibit greater neophobia than others, but observations of the zebra finches gave no impression that they were avoiding the area around the NC or NCO treatments. A number of birds that were not eating the NC or NCO treatments perched on the presentation cups during their trials. Clearly neophobia (both of the experimental equipment and of the coloured food) was no longer in operation. This being the case, we need another way of describing the birds' reactions towards the NC and NCO treatments. As discussed in section 1.4, I will use the term dietary conservatism (DC) (Marples *et al.* 1998) to describe this situation, i.e. the food is familiar, but the birds refuse to contact or sample it.

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If we consider the relative roles of neophobia and DC, we can see how multimodal defences may help protect prey species. The zebra finches showed increased neophobia (latency to contact food) towards the coloured treatments (Fig. 2.1). However, there was no increased neophobia shown towards the NCO treatment compared with the NC treatment. So neophobia as described previously (section 1.2.1), was not in this instance the important part of a multimodally novel prey's defence. The zebra finches also showed increased DC (latency to eat food), towards the coloured treatments (Fig. 2.1). Unlike the neophobic responses of the zebra finches, the DC 'effect' was greater for the NCO treatment than the NC treatment. This food was perfectly palatable, and yet the zebra finches chose not to eat it. This suggests it is the DC aspect of a predator's reaction towards multimodally novel prey that enhances that prey's protection, rather than the predator's neophobic responses. Previous authors have considered neophobia and DC as a combined response towards novelty (Barnett 1958; Coppinger 1969; Coppinger 1970; Jones 1987; Greenberg 1990b; Rowe & Guilford 1999b). Such authors might have reasonably expected the various components of a predator's behaviour to be of the same intensity. Here is a clear demonstration of the neophobic and DC aspects of an animal varying in different ways when confronted with novel foods. It would seem appropriate that neophobia and DC should be considered discrete processes. An animal's reaction towards new food can be more fully explained when these two processes are considered individually.

We might reasonably assume that wild birds would show greater neophobic and DC responses towards an NCO treatment than the laboratory birds considered here (see section 1.2.4). Zebra finches will readily feed on small larvae (Sillén-Tullberg 1985), so initially, at least, there appears to be a strong advantage for a larval prey item to acquire a novel colour and an accompanying novel odour, with respect to its protection from zebra finches. If a novel coloured morph were to arise in an established population at a low density, it appears likely that experienced predators would not be motivated to attack it. This contrasts with studies in the wild (Avery & Mason 1997; Nelms & Avery 1997), where abundant 'prey' with multimodal defences appear to require an accompanying cost (toxicity or other unpleasant consequence of exposure) to deter predators. It appears that the experience of the predators and the relative abundance of the prey are both important issues when considering predator/prey interactions.

In the laboratory, there appeared to be greatly reduced neophobia and DC towards previously incorporated foods for at least eight months. One might reasonably expect wild

birds to show similar trends in their behaviour. The average lifespan of the zebra finch is actually less than a year in the wild, owing to high infant mortality (Zann 1996). Those individuals that do survive from one year to the next should retain a memory of their previously incorporated foods. In the laboratory, first contact on re-exposure to a previously incorporated food was immediately followed by first consumption of that food. So the DC part of the birds' latency to sample novel coloured food had become negligible. For the birds considered here, it appears that DC can be entirely abolished by previous experience of that food type.

2.6 <u>Conclusions:</u>

Laboratory-held zebra finches reacted in the same way towards the novel colour and novel odour cues as laboratory-held chicks have done in previous experiments. The intensity of the cues used in this experiment matched those of the previous chick experiments. This strengthens the case for using chicks as general bird models within laboratory situations. A number of further observations about the behaviour of zebra finches were noted:

- Zebra finches used odour to assess the acceptability of novel coloured food.
- Zebra finches showed an equal neophobic response towards food of novel colour and food of novel colour and novel odour.
- Zebra finches showed greater DC towards food of novel colour and novel odour than to food of novel colour.
- Novel colours of familiar foods were sufficient to deter experienced zebra finches from feeding on them for a considerable amount of time.
- Memory for incorporated foods lasted at least eight months for zebra finches in captivity.

Chapter 3

The responses of adult solitary wild passerines to novel foods with combinations of novel colour and novel odour

3.1 <u>Introduction:</u>

In the laboratory, a number of workers have shown that the latency to attack food of novel colour is greater than that for familiar food, in both domestic (Ingalls 1993; Marples & Roper 1996) and wild-caught (Pawlina & Proulx 1996; Lindström *et al.* 1999a) species. This effect appears even greater in the wild. Work with wild blackbirds has shown that they may take very long periods of time to incorporate a familiar food of novel colour while they continue to eat the familiar colour of that food (Marples *et al.* 1998). These long latencies occur despite both foods being equally palatable. Such findings suggest that the responses of wild birds in the field differ significantly from their responses in the laboratory. It is therefore important to conduct wild studies in order to establish wild behaviour.

Marples *et al.* (1998), in their work with wild blackbirds and robins, describe their birds as displaying dietary conservatism (DC) when offered a food of novel colour (section 1.4). Some of the birds, having been presented with a palatable, novel coloured food, took a long time to sample it. However, once sampled, the food was usually accepted and eaten on all subsequent presentations. They distinguished this process from neophobia (Barnett 1958) by the birds' reactions to the food. Marples *et al.* (1998) found that while the birds were continuing to select the familiar food during the trials, they were showing no fearful reactions to the presence of the new colour.

The experiments outlined here were designed to explore the protection offered to a newly evolved morph within an established, predated population, and more particularly whether wild passerines are sensitive to a novel odour either alone or in combination with a novel colour. The importance of novel odour and novel colour cues can then be related to both neophobia and DC in wild populations.

The experimental procedures listed below are divided into two sections. The first procedure was employed during the 1998 field season. It was subsequently modified, and this revised protocol was used for the 1999 and 2000 field seasons.

3.2 <u>Protocol 1:</u>

The trials were conducted in Archbishop Ryan Park in Merrion Square, Dublin. This area had previously been identified as a site containing a high population of blackbirds

and robins. Before testing began, extensive trapping of the birds ensured that the majority of the target species were colour ringed. This allowed simple individual identification during the novel food presentations.

The birds were trained to come to pre-determined feeding areas using a mixed food containing a variety of seeds and nuts, as well as bread and pastry baits. I advertised my presence by using a signal whistle, which I repeated when any target individual was feeding at the site. The target birds were then weaned onto an exclusive diet of artificial pastry prey (70g flour, 30g lard and 10 ml water). These prey were 1cm x 0.5cm x approx. 0.15cm in size. Once accepting the training food, the birds were then trained to take the pastry prey from the presentation dishes. Each dish was produced from a standard plastic petri dish (9cm diameter). The larger diameter section was used as a base, and accommodated a filter paper that was soaked with 0.75ml of the appropriate olfactant (if no odour was required, water was used instead). The smaller diameter section of the dish was perforated with eight equally spaced lines of small holes radiating from the centre. This perforated section was sanded with fine sandpaper to reduce reflections on its surfaces, and the underside was painted black to provide a contrasting background for the presentation of the baits. Four small pads were attached to the underside of this section, and it was then placed inside the larger section. The small pads separated the two sections, and prevented transfer of the olfactant to the baits. This guaranteed the presence of odour without any flavouring of the baits (Järvi & Wiklund 1984).

Once the birds were trained to eat the control food from the dishes, they were then tested with the experimental treatments. These treatments were; Control (C) - the training food; Novel Odour (NO) - the training food in the presence of an unfamiliar odour; Novel Colour (NC) - a coloured version of the training food, and Doubly Novel (NCO) - a coloured version of the training food in the presence of an unfamiliar odour. The three new treatments were designed to be three new 'morphs' of the original training food.

Colour and odour combinations were chosen to mimic natural defence strategies; Green and almond to replicate a defended plant, and hence a potential invertebrate predator of that plant (Eisner 1970; Eisner & Meinwald 1995; Camara 1997), and red and pyrazine to reproduce the colour and odour cues used by ladybirds (Moore *et al.* 1990). The park was divided into two halves (east and west) in such a way that there were approximately even numbers of defended territories (Cramp 1998) in each half. In the east side the birds were presented with the green/almond combination, and the west side the red/pyrazine.

Colours were added to the pastry by using suitable dilutions of culinary food dyes. Colours and odours were obtained from O'Brien's Ingredients (O'Brien House, Ballymoss Road, Sandyford Industrial Estate, Dublin 18, Ireland). The colours were diluted with water to give a 50% solution of O'Brien's Green (90) and a 25% solution of O'Brien's Christmas Red (4R). Judging by eye, these concentrations were considered to have equal colour saturations. Subsequent spectrographic analysis of these baits (Fig. 3.1) showed this judgement to be accurate. Figure 3.1 demonstrates that all the bait types showed similar reflectance in their respective colour ranges, and reflectance was limited to their appropriate colour. The odours used were Quest Chocolate Almond flavouring in undiluted form, and a 0.0003% solution of pyrazine (2-methoxy-3-isopropylpyrazine from Pyrazine Specialties Inc. P.O. Box 6933, Atlanta, Georgia 30315, USA). Previous experience has shown these to be similar to each other in intensity (Marples & Roper 1996).

For each presentation two baits of each treatment were made available, giving a total of eight food items. The baits were arranged in each dish such that any pair was either in a vertical or horizontal line with respect to the observer. The dishes were presented to the birds in a two by two matrix (Fig. 3.2), between 6 to 8cm apart, with the NC and NCO treatments at opposing corners. This presented the birds with a bait density of approximately 140 baits m^{-2}. Opposing corners shared the same bait orientation. After each presentation the dishes were rotated by one position clockwise, and the orientation of the baits was changed. This technique was adopted to change the perspective of the presentations to the feeding bird, as a number of individuals often approached the dishes from the same direction.

The number of presentations was limited such that no individual bird was allowed more than three presentations at any particular feeding site. This allowed individual birds to have more than three presentations on any particular day, but they had to change their feeding site in order to do so.

A record was kept of each bird's food choices at each presentation. This record noted the number of baits, as well as the order in which they were taken. "Contact" with a treatment was recorded if a bird manipulated a bait, even if the bait was not eaten. "Incorporation" of a treatment was achieved if that treatment was consumed on three consecutive presentations. The number recorded was the first of these presentations, as this was the point at which the incorporation process began.
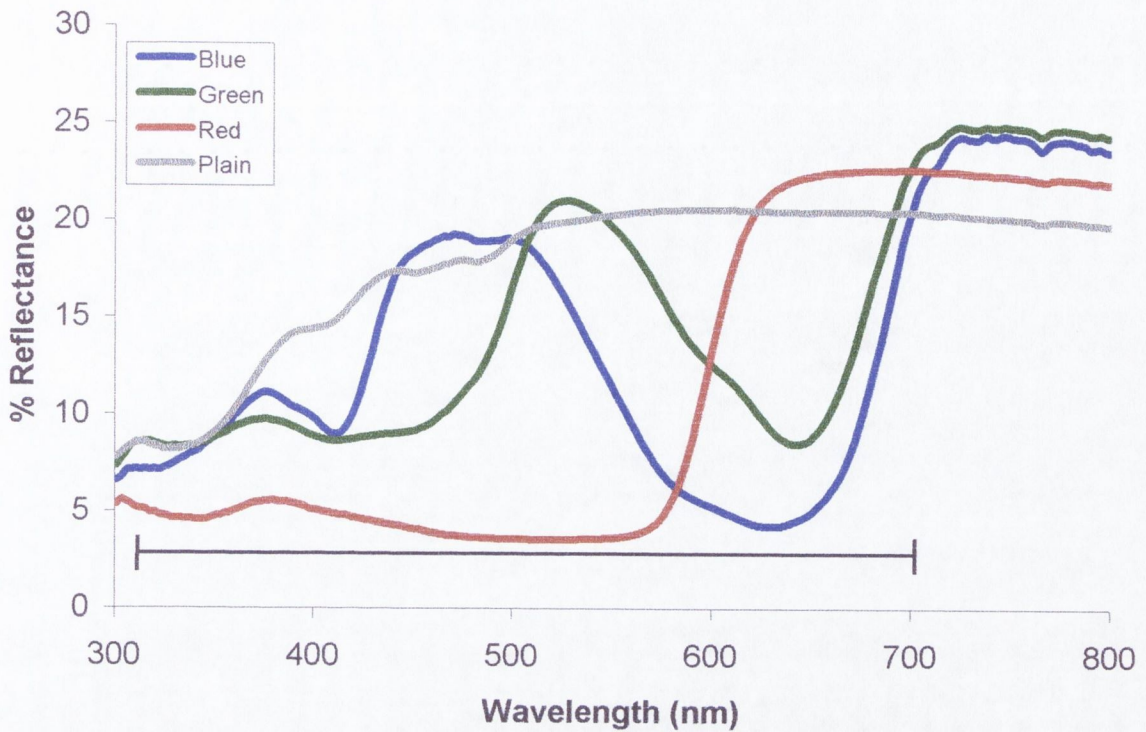
Figure 3.1. Reflectance spectrograms of the four bait colours presented to solitary wild birds over the 1999 and 2000 field seasons, showing the visual range of birds for comparison.
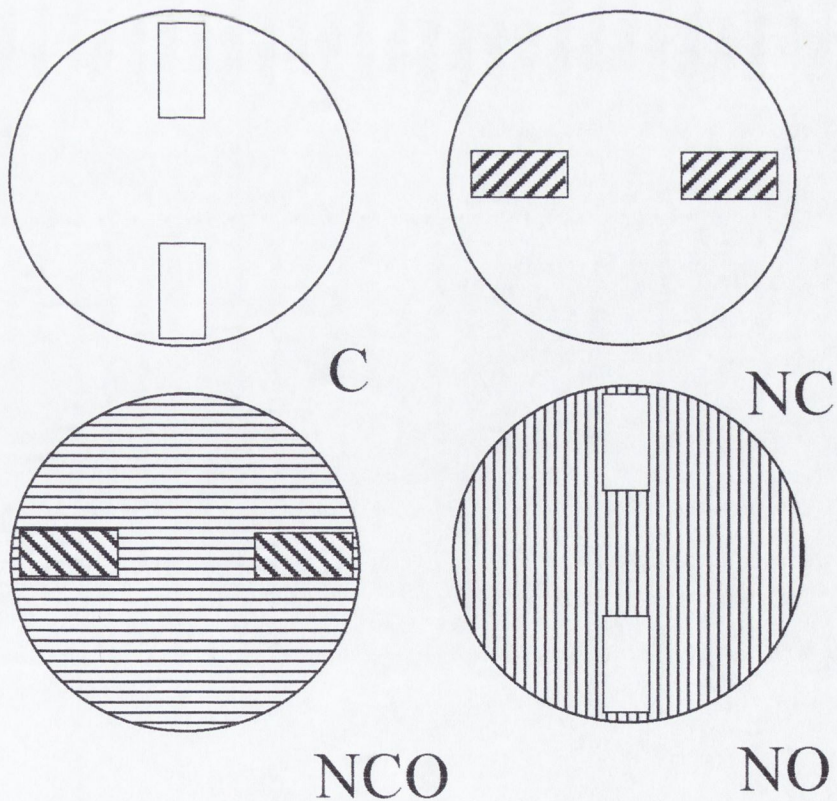
Figure 3.2. Diagram of dish and bait arrangements, showing relative positions and orientations. Control baits (C) and Novel Odour baits (NO) were uncoloured. Novel Colour baits (NC) and Doubly Novel baits (NCO) were coloured (shading of baits). NO and NCO baits were offered in the presence of a novel odour (shading of dishes).

The data were collected over a field season from 9th July 1998 to 1st September 1999.

3.3 <u>Data Analysis:</u>

As the latencies did not produce a normal distribution, the latency values were natural log-transformed to conform to the requirements of parametric data analysis (Shapiro-Wilk test: before conversion: $p <= 0.01$, after conversion: $p >= 0.20$).

Confidence intervals of the preference scores for the familiar coloured (FC) baits were calculated to allow determination of distinction between treatments (see appendix 2). Datasets were derived for birds eating three treatments (six baits) and birds eating all four treatments (eight baits). This gave a series of eight values. These values are shown in Table 3.1.

	Six Baits		Eight Baits	
	5 point	10 point	5 point	10 point
mean	0	0	0	0
95% C.I.	1.86	1.31	2.45	1.73
99% C.I.	2.44	1.73	3.22	2.28

Table 3.1. The means and 95% and 99% confidence intervals calculated for birds eating six or eight baits, based on five-point or ten-point moving averages.

Plots of the preference scores of experimental subjects were formatted using Microsoft Excel. Trend lines were added to datasets to show five point or ten point moving averages. Five point moving averages were used for smaller datasets, and ten point moving averages for larger ones. Other statistical tests were performed on SPSS© v10.0.5 (SPSS Inc. 1989 - 1999).

3.4 <u>Results 1:</u>

Any individual presentation lasted less than a minute. This included the approach by the bird to the dishes, an assessment of the food available, and subsequent selection of those baits. After consumption of the baits some birds would recheck the dishes they had just eaten from, before retreating from the feeding site. There were occasionally

interactions between marked birds at the feeding stations. The vast majority of these incidents were by robins darting in to a dish, removing a bait, and retreating to cover before a blackbird had selected any baits. When this happened, I had to prevent the blackbird from arriving at the dishes, replace the missing bait, and await the blackbird's return. Conspecific interactions occurred more frequently during the breeding season. On these occasions food was very rarely taken, but the feeding dishes were treated as an important part of disputed territory. On a few occasions I observed 'resource-based mate guarding', where male blackbirds appeared to defend the area around the feeding dishes while the females fed. On all of these occasions great care was taken not to allow any bird to approach the dishes without a full complement of test baits.

Data were collected on 10 robins and 17 blackbirds. All of the robins (9 out of 9), which had received sufficient presentations to do so, contacted and incorporated the C and NO treatments. No robins contacted either the NC or NCO treatments, however, it was still possible to calculate the 'minimum possible values' (MPV scores – see section 2.4) for contact and incorporation of these treatments. These values are simply the total number of presentations plus one. Using these MPV scores it was possible to analyse the robins' preferences with regard to the various treatments. Analysis showed that colour had a main effect for both contact and incorporation (repeated measures ANOVA: $F_{1, 8} = 81.2$, $p < 0.001$, $F_{1, 7} = 63.4$, $p < 0.001$ respectively), but odour alone had no effect on contact or incorporation. It was not possible to assess any interaction between colour and odour, as no robins contacted or incorporated either colour treatment.

All of the blackbirds (16 out of 16), which had received sufficient presentations to do so, contacted and incorporated the C and NO treatments. Five blackbirds contacted (3 females and 2 males) and two incorporated (1 female and 1 male) the NC treatment. Five blackbirds contacted (4 males and 1 female) and two incorporated (1 female and 1 male) the NCO treatment. As with the robins, MPV scores were used for comparison of the treatments (see appendix 3). Again, colour had a main effect for both contact and incorporation of the food (repeated measures ANOVA 95% CI: $F_{1, 16} = 149.1$, $p < 0.001$, $F_{1, 16} = 150.5$, $p < 0.001$ respectively), but there was no effect of odour or interaction of colour and odour on either contact or incorporation.

If we examine the birds that incorporated the coloured treatments, we can follow their preferences for the different treatments as they incorporate them into their diets. When the data were recorded the first bait a bird ate (or manipulated) was recorded as choice 1,

the second as choice 2 *et cetera*. This meant that preferred foods had lower choice scores, and less preferred foods had higher choice scores. As none of the blackbirds showed any preference between the C and NO treatments, these have been combined to produce a familiar colour treatment (FC). All birds incorporated the C and NO treatments very quickly (if not instantaneously), so all of the birds were eating at least four baits for most presentations. As there was no preference for either the C or NO treatments (as we have already seen), and no avoidance of them, then the average choice score for them should be 2.5, that is $(1+2+3+4)/4 = 2.5$. Similarly, when a bird was eating all of the treatments, they were eating eight baits. If there was no preference for the baits, then the average choice score for them was 4.5, that is $(1+2+3+4+5+6+7+8)/8 = 4.5$.

In figure 3.3, we can see that when the female blackbird GBL (FBGBL) started to eat the two coloured treatments (NC – green, NCO – green and almond) they were selected after the FC treatment baits. At presentation 15 the NC line crosses the FC line. I suggest that the bird was no longer showing a preference for the NC treatment over the FC treatment at this point. We can confirm this by plotting the preference scores for the coloured treatments (NC and NCO) over the FC treatment (i.e. mean NC score minus mean FC score, and mean NCO score minus mean FC score) for each presentation. These plots are given in figure 3.4. This figure gives the 95% and 99% CIs calculated in appendix 2. While treatment preference scores exceed these values, the bird is showing a preference for the familiar food. Once the treatment preference scores fall below these values, the bird is no longer showing a preference. So when the NC and NCO treatments were first eaten the bird was distinguishing these treatments from FC, as both preference score lines lie outside the 99% CI of 3.22 (Table 3.1). At presentation 15, the preference score for the NC treatment crossed the 95% CI, and stayed within its bounds for the duration of the field season (Fig. 3.4). So FBGBL was no longer distinguishing between the NC and FC baits. However, the line representing the NCO preference remained consistently above zero on the y-axis. This demonstrated that FBGBL maintained a distinction between the FC and NCO baits, and a preference for FC.

Let us consider the other bird that incorporated coloured food, MBRRL (a male blackbird) (Figs. 3.5 and 3.6). As with FBGBL, when MBRRL started to eat the two coloured treatments (NC - red, NCO – red and pyrazine) they were selected after the FC treatment baits (Fig. 3.5). At presentation 16 the NC line crossed the FC line, and at presentation 23 the NCO line crossed the FC line. If we investigate MBRRL's preferences

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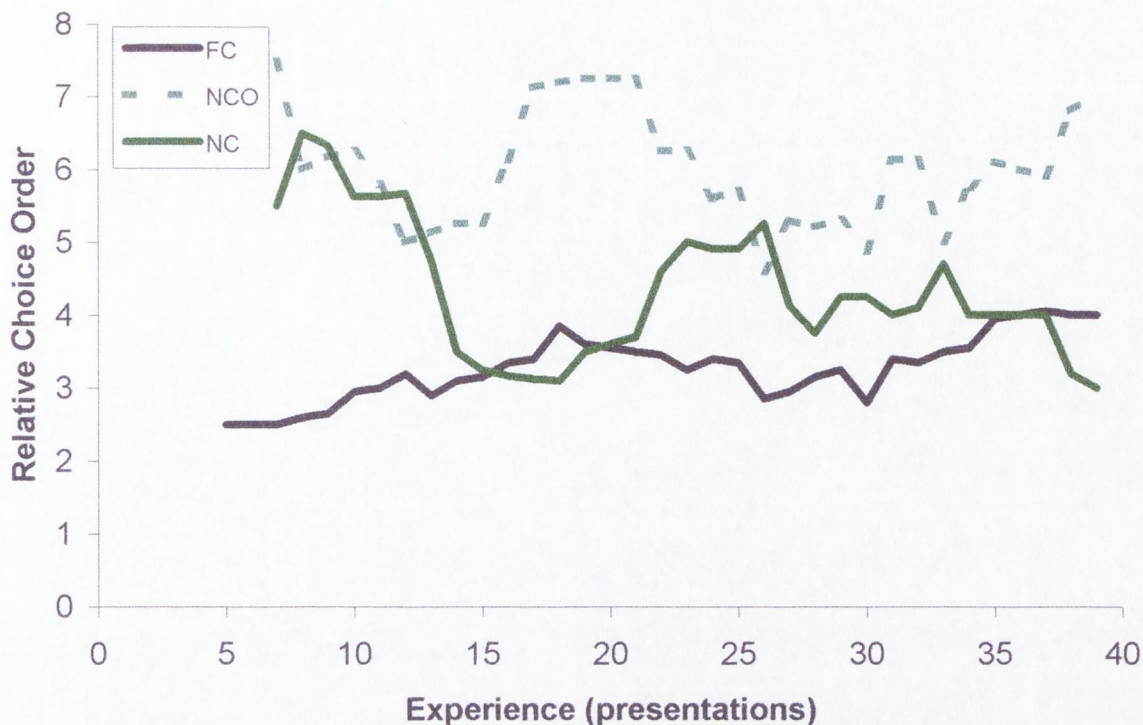
Figure 3.3. The incorporation process of green coloured food by a female blackbird (FBGBL) in the presence (NCO - the green dotted line) and absence (NC - the unbroken green line) of almond odour. The previously incorporated food of familiar colour (FC) is shown as an unbroken black line.The lines shown are five point moving averages.
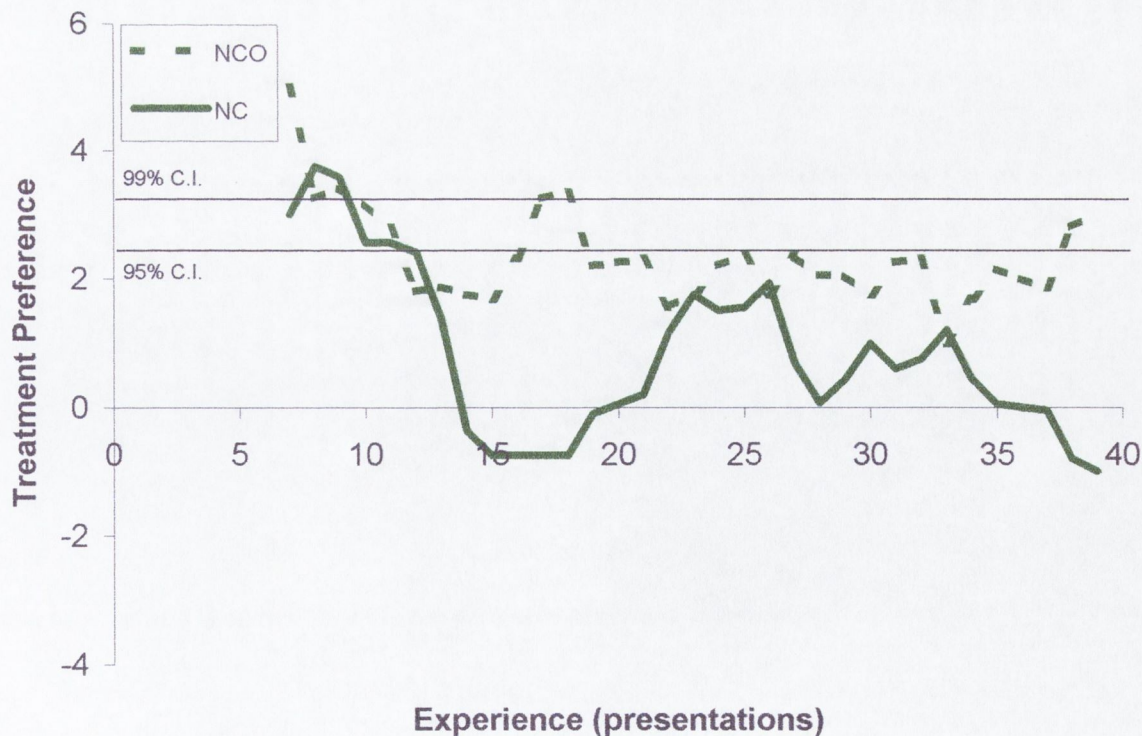
Figure 3.4. The dietary incorporation of a novel coloured food by a female blackbird (FBGBL) over the 1999 field season. NC (green line) represents novel green food, and NCO (dotted green line) represents novel green food in the presence of a novel almond odour. Both lines are shown as five point moving averages. (For calcualtion of preference score see text.)
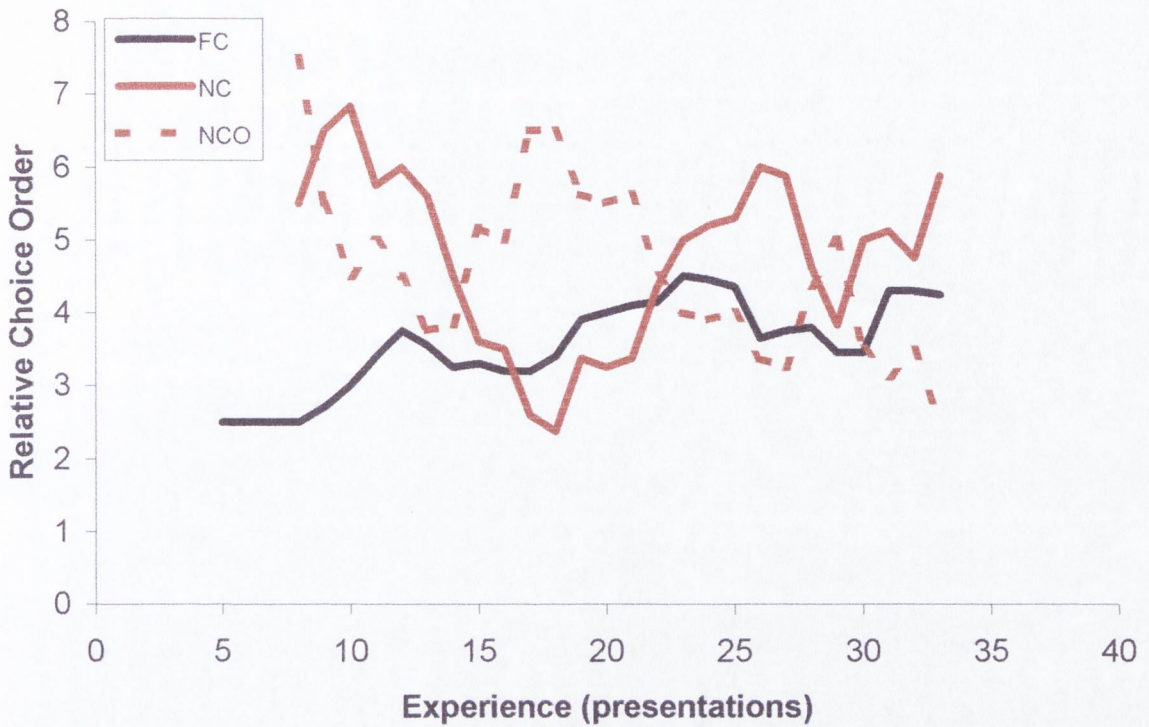
Figure 3.5. The incorporation process of red coloured food by a male blackbird (MBRRL) in the presence (NCO - the red dotted line) and absence (NC - the unbroken red line) of pyrazine odour. The previously incorporated food of familiar colour (FC) is shown as an unbroken black line. The lines shown are five point moving averages.
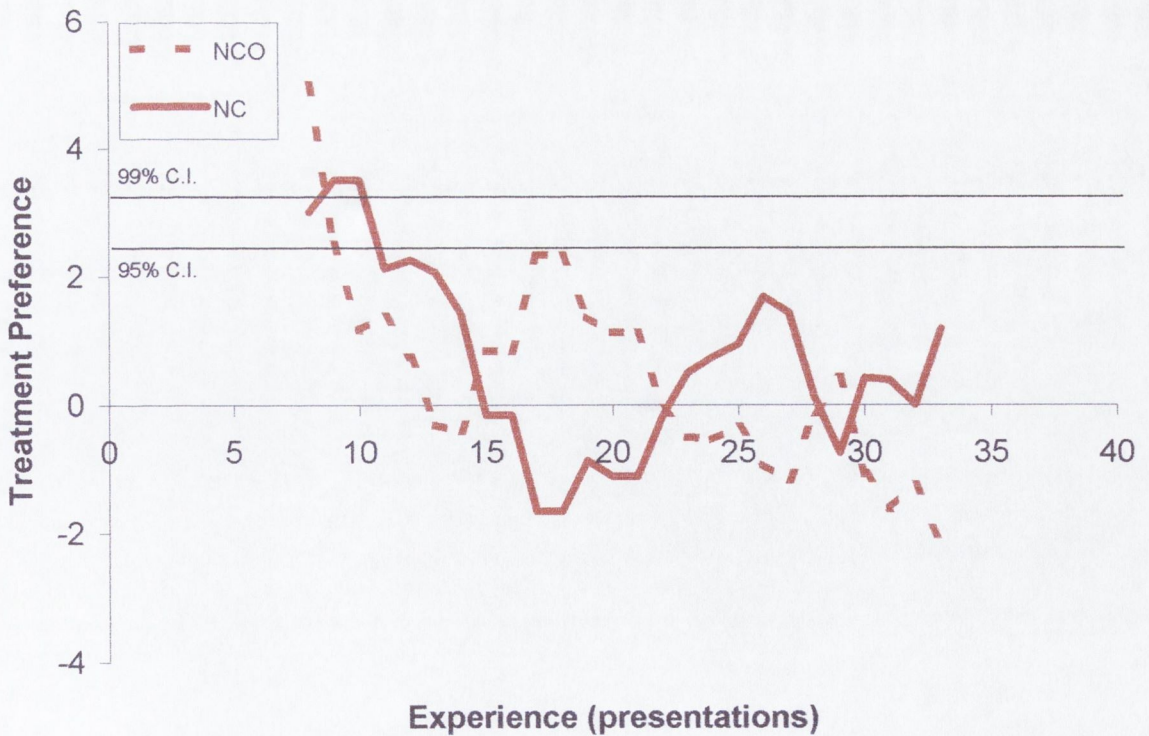
Figure 3.6. The dietary incorporation of a novel coloured food by a male blackbird (MBRRL) over the 1999 field season. NC (red line) represents novel red food, and NCO (dotted red line) represents novel red food in the presence of a novel pyrazine odour. Both lines are shown as five point moving averages. (For calculation of preference score see text.)

for the coloured treatments with a preference plot (Fig. 3.6), we can see that both the NC and NCO lines maintained their positions inside the 95% CI after presentation 11. MBRRL was not distinguishing either the NC or NCO treatments from the FC treatment from this point on.

3.5 <u>Discussion 1:</u>

Primarily, we can make two assertions. Both blackbirds and robins were slow to investigate a novel colour of a familiar food type. Neither blackbirds nor robins appear to be deterred by an unfamiliar odour of a familiar coloured food. The interaction of novel colour and odour cues appears less clear. FBGBL could distinguish the NC and NCO treatments, even when they were the same colour (Figs. 3.3 & 3.4). This was demonstrated by the more cautious approach taken to the NCO treatment. MBRRL failed to distinguish the treatments in the same way, although the way in which the NC and NCO lines mirrored each other (one increased in the same way that the other decreased) from presentation 18 (Fig. 3.6) suggests that the bird was managing to separate them conceptually. By my original criterion, FBGBL incorporated both the NC and NCO treatments simultaneously, but appears to have treated them differently none the less. This does suggest that the original condition I set (i.e. eating a treatment on three successive presentations) was a somewhat simplistic assessment of dietary incorporation. I would like to refer to my original criterion of incorporation as differential incorporation from now on. Differential incorporation is a situation where a treatment is being eaten, but where FC food is being eaten in preference to that treatment (e.g. the behaviour of FBGBL towards the NCO treatment (Figs. 3.3 and 3.4)). This allows for a further definition of non-differential incorporation. Non-differential incorporation is a situation where a treatment is being eaten, but there is no preference for FC food (e.g. the behaviour of FBGBL towards the NC treatment after presentation 14 (Figs. 3.3 & 3.4)). Using this new terminology, we can say that FBGBL demonstrated only differential incorporation of the NCO treatment, but non-differential incorporation of the NC treatment by the end of the field season. The trends of the NC and NCO lines for MBRRL (Figs. 3.5 & 3.6) appear to be the same as that of the NC line for FBGBL (Fig. 3.3 & 3.4). On initial consumption, the new treatment was less preferred (i.e. with a higher choice order score), but that preference was not maintained. So we can say that MBRRL demonstrated non-differential incorporation of both the NC and

NCO treatments for the 1998 season. FBGBL and MBRRL reacted differently to the bimodal cue of novel colour and novel odour. It is not clear if this was due to the different colours and odours used for the different birds, or a difference in the individual behaviour of these birds. There was a clear indication that one of the two birds that incorporated the NCO treatment (FBGBL) could separate it from the NC treatment. This suggests that this bird, at least, was sensitive to almond odour.

As with the zebra finches (chapter 2), the trained blackbirds gave no impression that they were avoiding the area around the novel coloured baits. A number of birds that were not eating novel coloured baits stood on the NC or NCO dishes, and sometimes even on the baits themselves, while selecting and eating the FC baits. These birds can be considered to have been demonstrating dietary conservatism (Marples *et al.* 1998) rather than neophobia.

A comparison of the number of robins that contacted novel coloured food (0 out of 10) and the number of blackbirds which did so (5 out of 17), might lead one to suggest that robins are more neophobic than blackbirds. I do not believe that these data can reliably inform us of this conclusion. As I mentioned previously, a number of robin records consisted more of 'raids' than of orderly feeding presentations. Robins and blackbirds were competing for the same food resource here. Blackbirds, being larger, were the more dominant, and easily defended the presentation sites against the smaller robins. As a result, the average time of a robin presentation was less than that of a blackbird presentation, and robins only took all baits of familiar colour on 17% of presentations in the 1998 field season. Blackbirds by comparison took all baits of familiar colour on 90% of presentations.

There seems to be a general process of dietary incorporation. This process appears to be multi-staged, and may incorporate both the phenomena of neophobia (latency to approach the new food (Brigham & Sibly 1999)) and dietary conservatism (latency to eat the new food (Marples *et al.* 1998)). Intriguingly, it appears that a bird is capable of eating a food type while still discriminating against it (see Figs. 3.3 & 3.4).

3.6 <u>Protocol 2</u>:

When protocol 1 was running during the 1998 field season, I began to be concerned about the maintenance of novelty of the stimuli during the experiment. I was particularly concerned that if a bird ate the NC treatment, and was already eating the NO treatment,

there would be very little novel about the NCO treatment. Those birds would already be familiar with both the odour and colour components of the NCO treatment. This was borne out by FBGBL and MBRRL who both started to eat the NC and NCO treatments at the same time. To rectify this potential problem, protocol 2 was devised. Here the colour and odour used in the NCO treatment were different from the NO and NC stimuli, in order to maintain the novelty of these cues. On this occasion different colour and odour combinations were used in opposite halves of the study area to control for individual effects of colour and odour. These colours and odours were also chosen to allow the maintenance of novelty of these cues to birds that had participated in the previous study (sections 3.2 and 3.4).

The same green and red colours were used, but a blue colour was added to this protocol (a 10% solution of O'Brien's Blue (FCF) – judged by eye to be similar in intensity to the red and green treatments). The spectrogram for this bait colour is included in Fig. 3.1. The odours used were Quest Chocolate Almond flavouring and O'Brien's Vanilla flavouring in undiluted form. Thus in the west side of the park the treatments were: NO = vanilla, NC = blue, and NCO = green and almond. In the east side of the park, the treatments were NO = almond, NC = red, and NCO = blue and vanilla.

Data recording techniques remained unchanged. These data were collected over two field seasons between 28th April 1999 and 10th September 2000.

3.7 Results 2:

Data were collected on 22 robins (Fig. 3.7) and 32 blackbirds (Fig. 3.8). These included some birds from Protocol 1. The majority of the robins (20 out of 21) that had received sufficient presentations to do so contacted and incorporated the C and NO treatments. Two robins contacted the NCO treatment, and one subsequently incorporated it. No robins contacted or incorporated the NC treatment. By deriving MPV scores (section 2.4) for contact and incorporation latencies of the NC and NCO treatments for birds that didn't produce them during the study, we can identify the important components of the robins' preferences. As with the first field season, we find that colour had a main effect for both contact and incorporation (repeated measures ANOVA 95% CI: $F_{1, 20} = 267.2$, $p < 0.001$, $F_{1, 19} = 170.4$, $p < 0.001$ respectively), but neither odour alone or in combination

Figure 3.7. Comparative latencies of robins to incorporate four food treatments in the 1999 and 2000 field seasons. The total number of presentations each individual has received is indicated by a dark, filled circle where a bird has yet to incorporate all treatments. This allows calculation of minimum possible incorporation values.
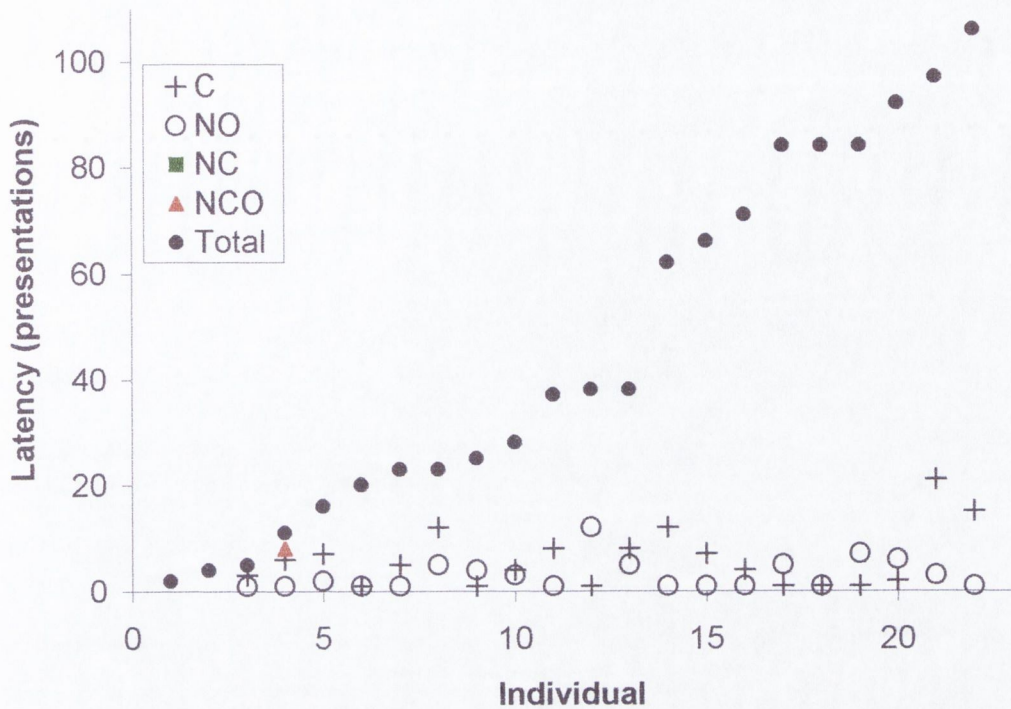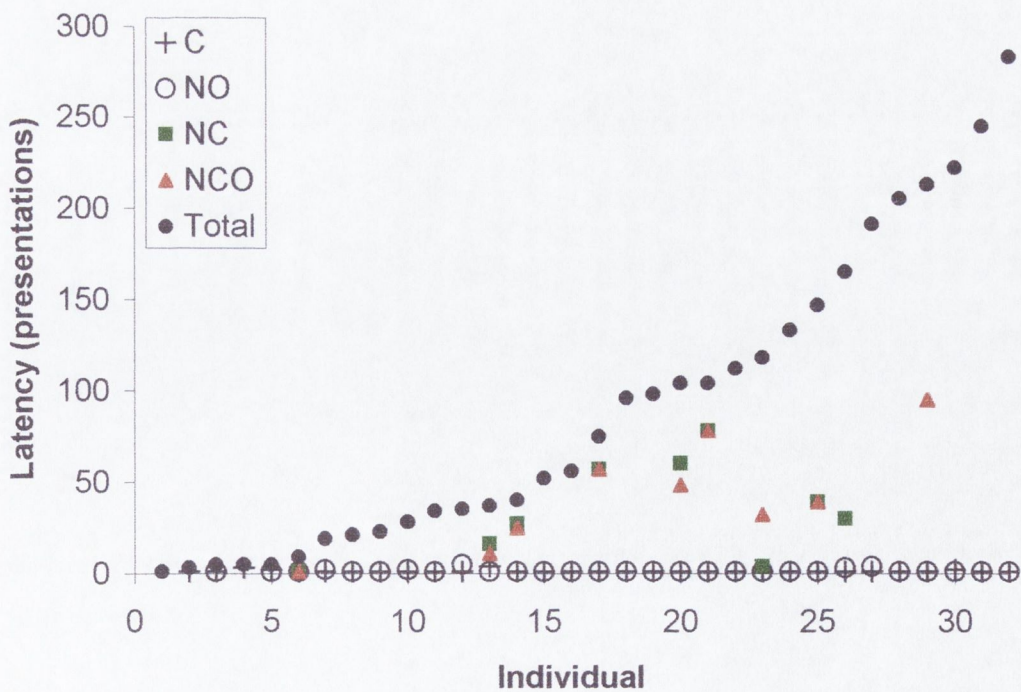

Figure 3.8. Comparative latencies of blackbirds to incorporate four food treatments in the 1999 and 2000 field seasons. The total number of presentations each individual has received is indicated by a dark, filled circle where a bird has yet to incorporate all treatments. This allows calculation of minimum possible incorporation values.

with colour had an effect on contact or incorporation. As only one robin actually incorporated the NCO treatment, it would be unfair to claim an interaction (or lack of it) between odour and colour.

Of the 31 blackbirds, which had received sufficient presentations to do so, all of them contacted and incorporated the C treatment. Furthermore all of them contacted and 29 incorporated the NO treatment. Sixteen blackbirds contacted (9 females, 6 males and 1 juvenile) and nine incorporated (5 females and 4 males) the NC treatment. Fourteen blackbirds contacted (8 females, 5 males and 1 juvenile) and nine incorporated (5 females and 4 males) the NCO treatment. We can see from figure 3.8 that there was a great deal of variation in the time taken to incorporate a novel coloured food. A few blackbirds had incorporated either the NC and or the NCO treatment after only 10 presentations, while other birds had yet to incorporate these treatments after 200 presentations or more. As with the robins, MPV scores were calculated to allow comparison of the treatments (see appendix 3). Colour had a main effect for both contact and incorporation of the food by blackbirds (repeated measures ANOVA 95% CI: $F_{1, 27} = 161.2$, $p < 0.001$, $F_{1, 27} = 296.7$, $p < 0.001$ respectively), but there was no effect of odour in either situation. Separate calculations using just the blackbirds that contacted or incorporated the novel colour treatments (NC and NCO) showed there was no interaction between colour and odour for either contact with ($F_{1, 7} = 1.00$, $p = 0.35$) or incorporation of ($F_{1, 7} = 0.57$, $p = 0.48$) the novel foods. There was also no significant difference among all of the four colour treatments (NC = red, NC = blue, NCO = blue and vanilla, NCO = green and almond) for either contact ($F_{3, 25} = 0.72$, $p = 0.55$) or incorporation ($F_{3, 14} = 0.92$, $p = 0.46$).

As the preference plots are useful in assessing the distinction of the treatments (see section 3.4), only these plots will be provided for the birds considered in this section. The blackbirds that incorporated coloured treatments can be split into three groups: There were those that incorporated one treatment (either NC or NCO), those that incorporated both, and naïve birds (which lacked the training process, and therefore had no 'familiar' colour). Figure 3.9 illustrates data for a female blackbird (FB4U6) that incorporated the NC treatment (red), but not the NCO treatment (blue and vanilla). FB4U6 first ate the NC treatment at presentation 16. The NC treatment was initially distinguished from the FC baits (i.e. differentially incorporated – see section 3.4), but at presentation 38 the treatment preference score decreased dramatically. From here on, the NC treatment preference line

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Figure 3.9. The dietary incorporation of a novel coloured food by a female blackbird (FB4U6) over the 1999 and 2000 field seasons. NC (red line) represents novel red food, and is shown as a ten point moving average. (For calculation of preference score see text.)
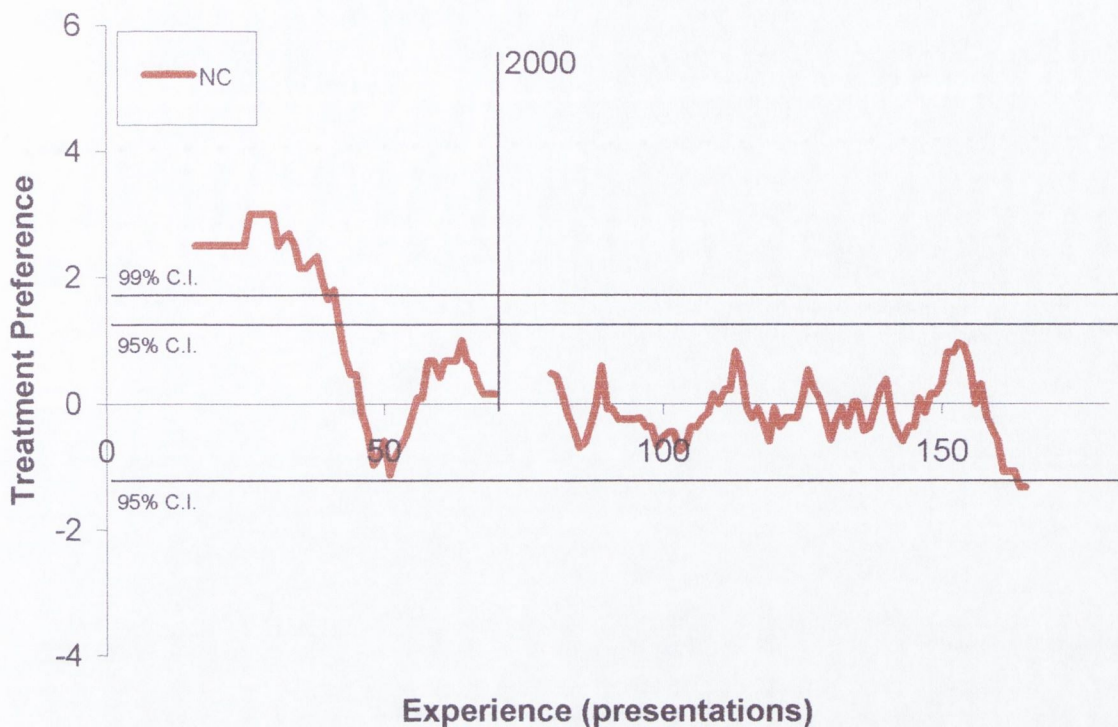

Figure 3.10. The dietary incorporation of a novel coloured food by a female blackbird (FBRRL) over the 1999 and 2000 field seasons. NCO (green line) represents novel green food in the presence of novel pyrazine odour, and is shown as a ten point moving average. (For calculation of preference score see text.)

remained inside the 95% CI line. FB4U6 had non-differentially incorporated the NC treatment (see section 3.4). At the end of the 2000 field season (presentation 165), it appeared that she might actually have been developing a preference for the NC treatment over the FC treatment, as the NC preference score line was just starting to cross the lower 95% CI line. FB4U6 was tested over two field seasons. Presentation 70 was on 25th July 1999 and presentation 71 was on 18th January 2000, a gap of just over six months. This gap is represented physically in the preference plot, as the preference values are displayed as ten point moving averages (i.e. they need ten values before they can generate their first score).

If we compare the preference score for NC at the end of the 1999 field season, and the beginning of the 2000 field season we can see that the preference score rose slightly (but not outside the 95% CI line) at the beginning of the new field season. None the less, FB4U6 continued to eat the NC treatment non-differentially. FB4U6 did not eat the NC treatment on first presentation, but DC towards it was greatly reduced (latencies to eat: 1999 = 16 presentations, 2000 = 2 presentations).

Figure 3.10 illustrates data for a female blackbird (FBRRL) that incorporated the NCO treatment (green and almond), but not the NC treatment (blue colour). The preference score for NCO remained high for the duration of the 1999 field season. So FBRRL maintained a distinction between the NCO and FC baits while continuing to eat both. FBRRL was also tested over two field seasons, with a gap of six months between. Presentation 145 marks the beginning of the 2000 field season. FBRRL continued her differential incorporation of the NC treatment (i.e. eating NC, but only after eating FC) in the 2000 field season. FBRRL ate the NCO treatment on the first presentation in 2000, so both neophobia and DC had been abolished, yet differential incorporation was maintained. There was a sharp drop in the preference score during the 2000 field season, but the trend was not continued, and the preference score rose sharply immediately afterwards. So for the majority of the 2000 season the NCO preference score was outside the 99% CI line, and so the bird was discriminating against this treatment.

Figure 3.11 shows data for the non-differential incorporation of the NC (blue) and NCO (green and almond) treatments by MBMWR (a male blackbird). The NCO treatment was first eaten at presentation 25, and the NC treatment was first eaten at presentation 27. The NCO treatment was non-differentially incorporated more rapidly than the NC treatment. The preference score for the NC treatment (Fig. 3.11) shows that at the end of

Figure 3.11. The dietary incorporation of novel coloured foods by a male blackbird (MBMWR) over the 1999 field season. NC (blue line) represents novel blue food, and NCO (green line) represents novel green food in the presence of a novel almond odour. Both lines are shown as five point moving averages. (For calculation of preference score see text.)
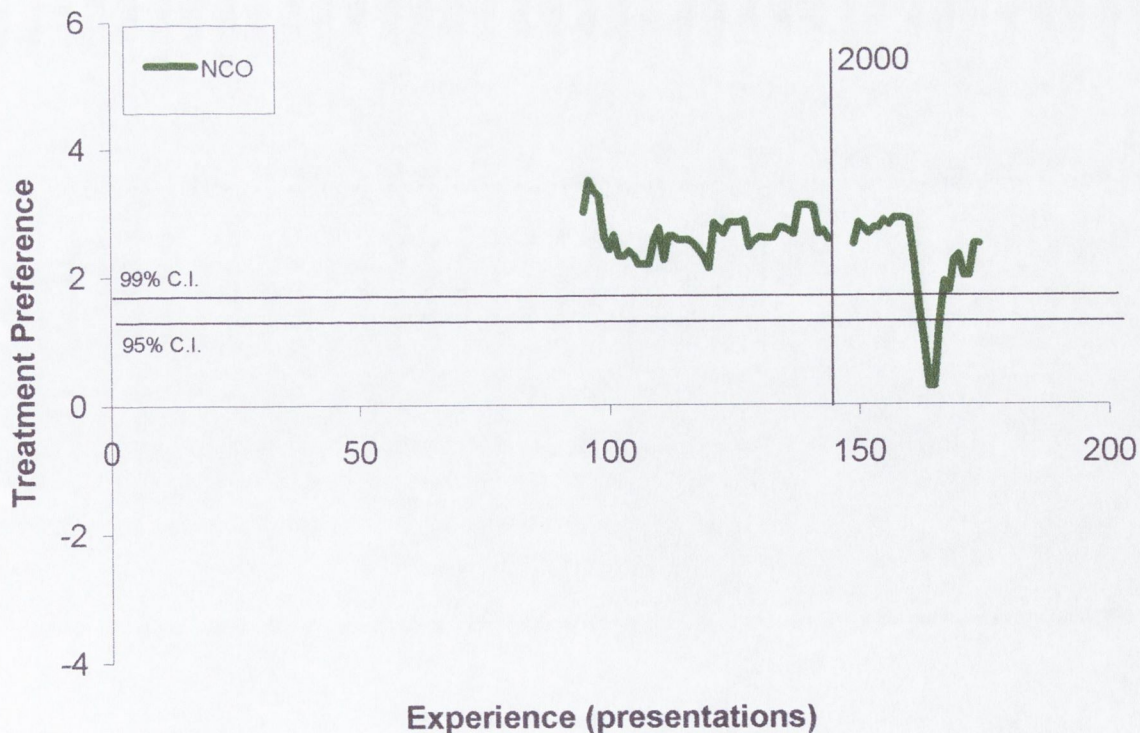

Figure 3.12. The dietary incorporation of a novel coloured food by a female blackbird (FB4US) over the 2000 field season. NCO (green line) represents novel green food in the presence of novel pyrazine odour, and is shown as a ten point moving average. (For calculation of preference score see text.)

the field season MBMWR was actually showing a slight preference for the NC treatment over the FC treatment. Sadly, the data is limited to the 1999 field season, as MBMWR was found dead at the beginning of the 2000 field season.

FB4US (a female blackbird) was relatively quick to non-differentially incorporate both the NC and NCO treatments (Figure 3.12). The preference score line for the NC treatment did wander outside the 95% CI line, but both the NC and NCO treatment preference score lines stayed within the 99% CI boundary after non-differential incorporation. All presentations to FB4US took place in the 2000 field season.

MBGRL (a male blackbird) was tested with three colours of food over two field seasons (Fig. 3.13). During the 2000 field season he attended the feeding sites very regularly. After he had incorporated the original coloured treatments allocated (NC – blue, NCO – green and almond), he was further tested with a second novel colour (NC2 – red). All three colour treatments were non-preferentially incorporated during the period of the experiment. Both the NC (blue) and NCO (green and almond) treatments were first eaten at presentation 78, but were non-differentially incorporated rapidly thereafter. The NC2 treatment (red) was also rapidly incorporated in a non-differential manner. The latency to eat this treatment (five presentations) was noticeably shorter than that to eat the original NC treatment (78 presentations).

MBRRL (Fig. 3.14) was also tested over two field seasons. MBRRL incorporated both the NC and NCO treatments shortly before the end of the 1999 field season (presentation 64). At the beginning of the 2000 field season both treatments were initially differentially incorporated, before being non-differentially incorporated. So MBRRL reacted differently to the NC and NCO treatments at the end of the 1999 season compared to the start of the 2000 season. MBRRL ate both the NC and NCO treatments on the second presentation in 2000, so had significantly reduced DC towards them from the previous year (1999 NC = 52, NCO = 54, 2000 NC = 7, NCO = 7).

Figure 3.15 shows data for a female blackbird (FB4U5) over two field seasons. During the 1999 field season the NCO treatment (blue and vanilla) was non-differentially incorporated (treatment preference score plot within 99% CI line), while the NC treatment (red) was only differentially incorporated (treatment preference score plot outside 99% CI line). At the beginning of the 2000 field season both treatments were initially differentially incorporated, but both were subsequently non-differentially incorporated. FB4U5 did not eat the NC treatment immediately at the beginning of the 2000 season, but latency (DC)

Figure 3.13. The dietary incorporation of novel coloured foods by a male blackbird (MBGRL) over the 1999 and 2000 field seasons. NC (blue line) represents novel blue food, NC2 (red line) represents novel red food and NCO (green line) represents novel green food in the presence of a novel almond odour. All lines are shown as five point moving averages. (For calculation of preference score see text.)
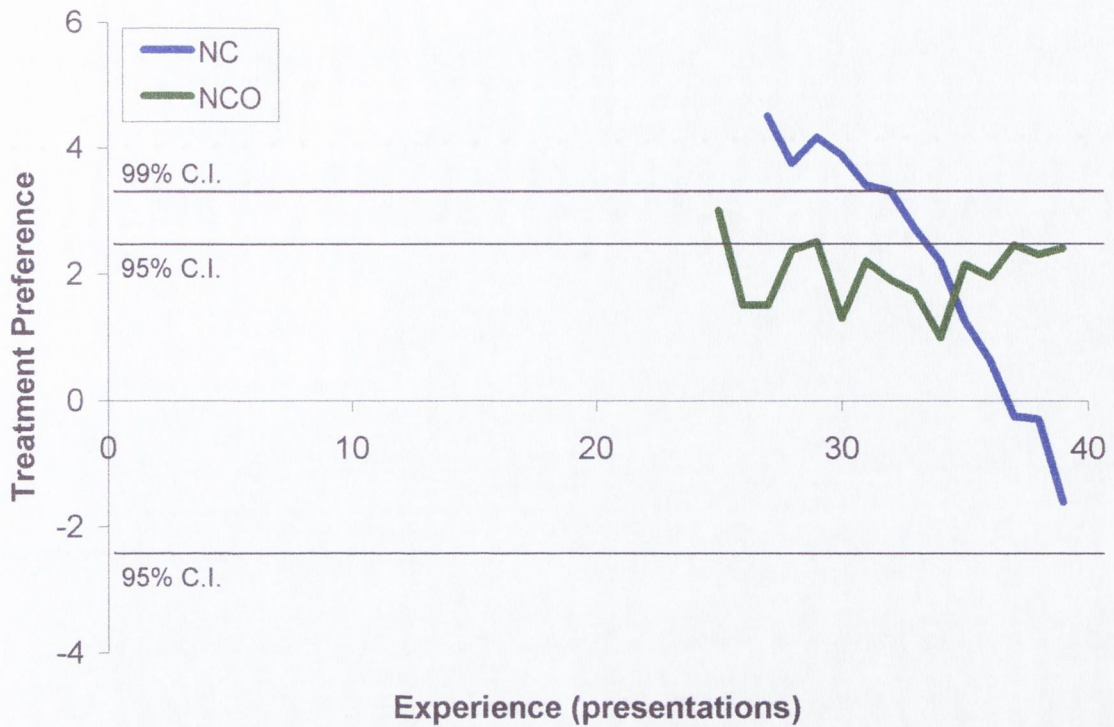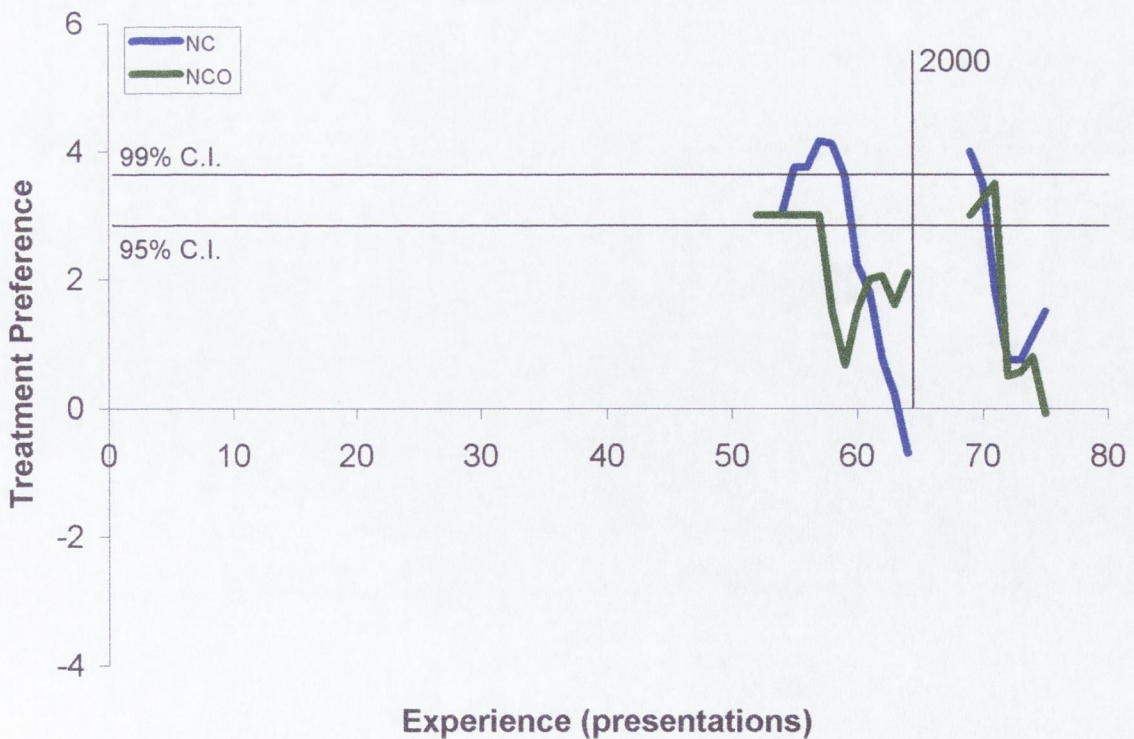

Figure 3.14. The dietary incorporation of novel coloured foods by a male blackbird (MBRRL) over the 1999 and 2000 field seasons. NC (blue food) represents novel blue food, and NCO (green line) represents novel green food in the presence of novel pyrazine odour. Both lines are shown as ten point moving averages. (For calculation of preference score see text.)
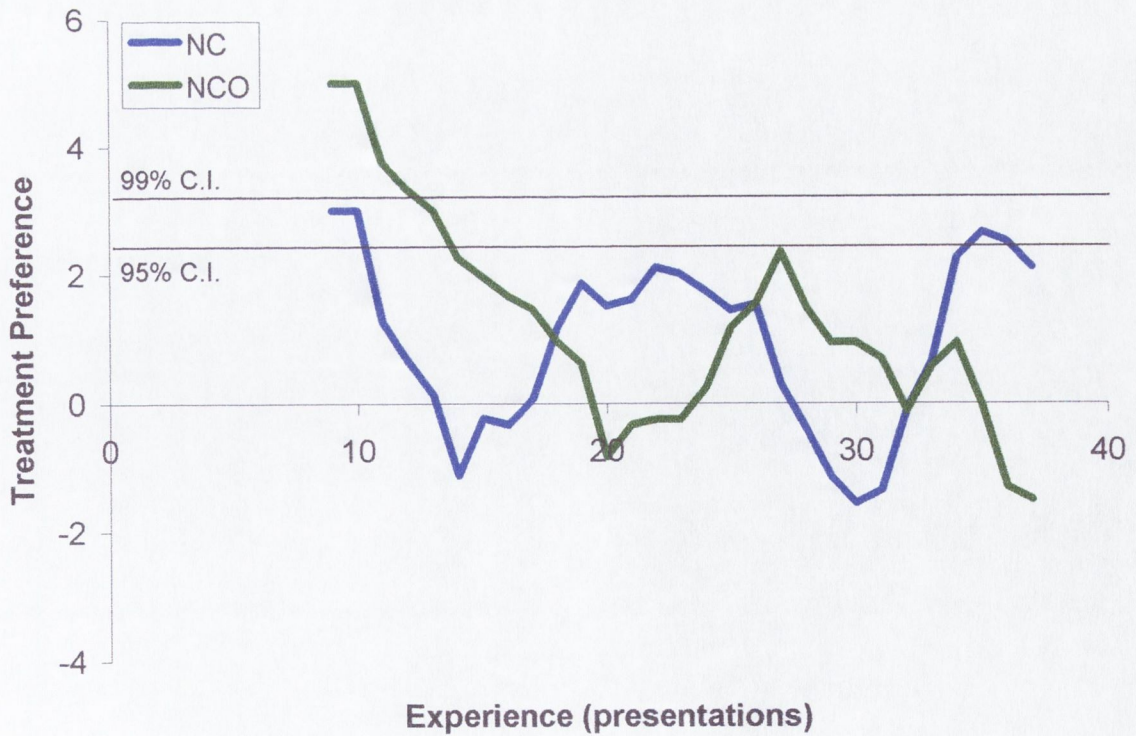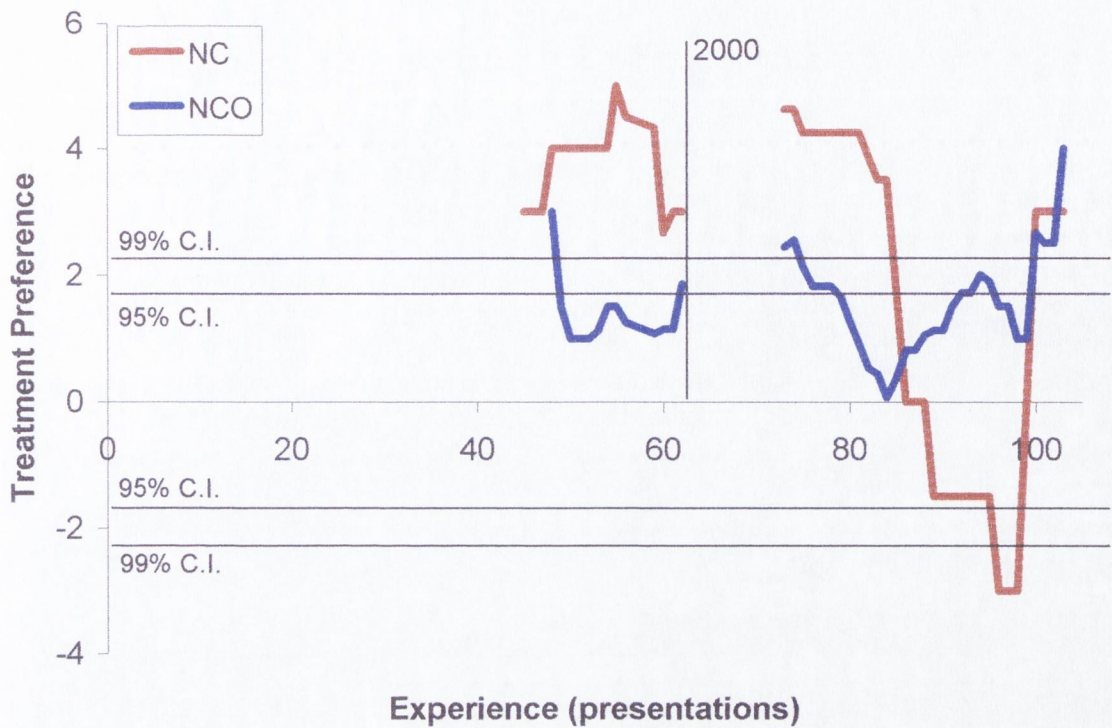
Figure 3.15. The dietary incorporation of novel coloured foods by a female blackbird (FB4U5) over the 1999 and 2000 field seasons. NC (red line) represents novel red food, NCO (blue line) represents novel blue food in the presence of a novel vanilla odour. Both lines are shown as ten point moving averages. (For calculation of preference score see text.)
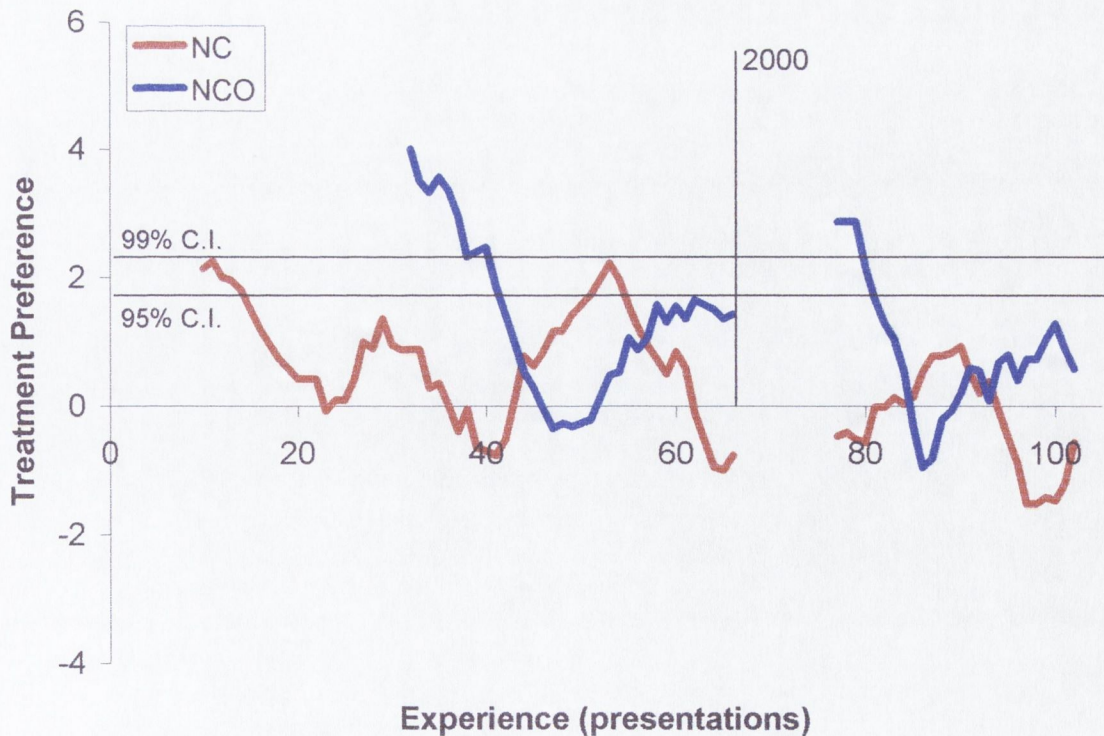
Figure 3.16. The dietary incorporation of novel coloured foods by a male blackbird (MB6U6) over the 1999 and 2000 field seasons. NC (red line) represents novel red food, NCO (blue line) represents novel blue food in the presence of a novel vanilla odour. Both lines are shown as ten point moving averages. (For calculation of preference score see text.)

was reduced compared to the previous season (latency to eat NC for 1999 season = 45 presentations, latency to eat NC for 2000 season = 9 presentations). The NCO treatment was eaten on the first presentation of the 2000 field season, and was also non-differentially incorporated more rapidly than the NC treatment. The NC treatment became preferable to the FC treatment at the end of the 2000 field season (preference score line fell below lower 99% CI line) for a short time.

The next bird for consideration is MB6U6 (a male blackbird) (Fig. 3.16). In the 1999 season MB6U6 non-differentially incorporated the NC treatment (red) first (presentation 10). The NCO treatment (blue and vanilla) was non-differentially incorporated subsequently (presentation 41). As the NCO preference score fell towards zero, so the NC preference score rose. The increase in the NC preference score almost took it outside the 99% CI line. MB6U6 showed a reinstatement of DC at the beginning of the 2000 field season, but this was reduced for both the NC treatment (1999 = 4, 2000 = 3), and the NCO treatment (1999 = 32, 2000 = 4). In the 2000 field season, the NC treatment was maintained at a non-differentially incorporated level, while the NCO treatment was only differentially incorporated at first. The rise in the preference score for the NC treatment was less this time, when the NCO treatment was non-differentially incorporated. MB6U6 appears to have been distinguishing between two types of baits that were non-differentially incorporated. Such a distinction would not have been guessed at from casual observations.

Figure 3.17 shows data for FBRWR (a female blackbird) over two field seasons. It appears that FBRWR did not make clear choices about the NC (blue) and NCO (green and almond) treatments in either field season, as in both cases non-differential incorporation was not maintained. The NCO treatment was non-differentially incorporated soon after first being eaten (presentation 40). At presentation 50, the treatment preference score for the NC treatment started to reduce. FBRWR was starting to non-differentially incorporate this food into her diet. This process affected her reaction towards the NCO food. As the NC treatment preference score decreased, the NCO treatment preference score increased, and eventually rose beyond the 99% CI line. This effect was not long lasting though, as shortly after non-differentially incorporating the NC treatment (preference line crossed the 99% CI line), the NCO treatment was non-differentially incorporated again. A similar situation can be seen for the 2000 field season, although the NCO treatment was maintained at its previous non-differentially incorporated level. The NC treatment was not eaten at the first
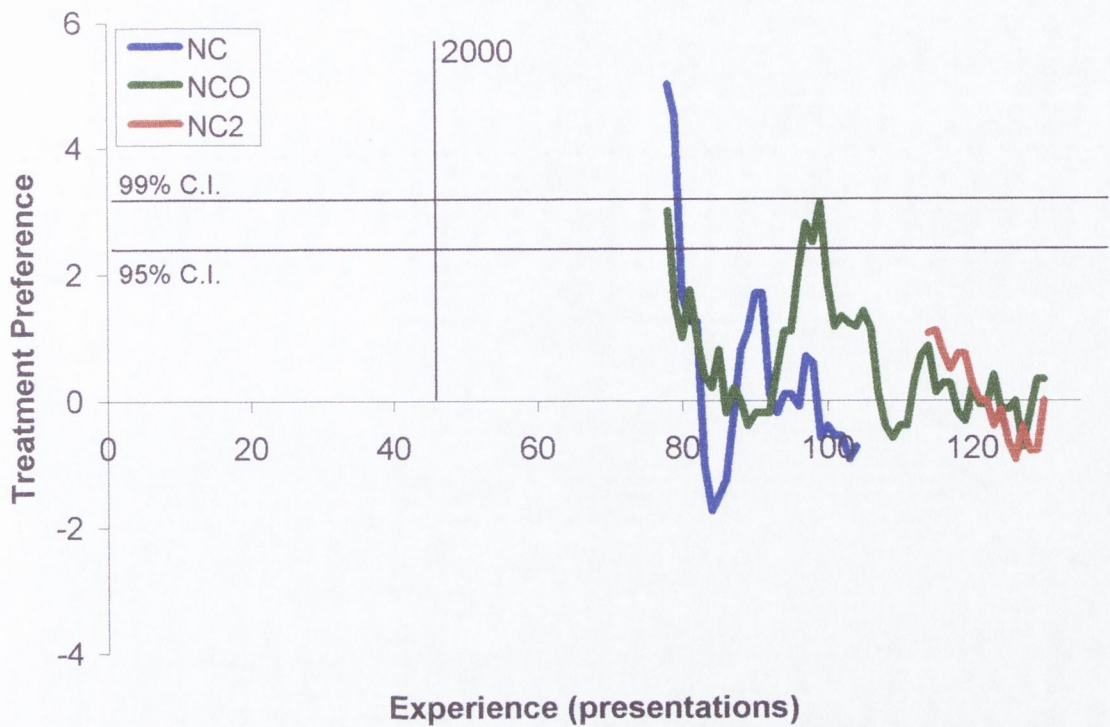
Figure 3.17. The dietary incorporation of novel coloured foods by a female blackbird (FBRWR) over the 1999 and 2000 field seasons. NC (blue line) represents novel blue food, NCO (green line) represents novel green food in the presence of a novel almond odour. Both lines are shown as ten point moving averages. (For calculation of preference score see text.)

presentation of the 2000 season, and was only differentially incorporated at first. FBRWR's reaction towards the NC treatment appears to have affected her preference for the NCO treatment, as the two preference scores mirror each other from about presentation 90 onwards. I believe that FBRWR (like MB6U6) was conceptually maintaining the distinction between the different coloured treatments even when they were non-differentially incorporated.

While all efforts were made to ensure that no bird entered the trials as a naïve predator, one adult blackbird (FB4U1) managed to do so. Two other birds were tested as naïve predators, but both of these were juveniles (R3JU3 & B3JU). The details of these birds are given below. It is interesting to contrast the behaviours of these birds with the fully trained group.

In figure 3.18 we see data for the only robin (R3JU3 – a juvenile) to incorporate food of a novel colour. The treatment incorporated was NCO (green and almond). The line generated from the preference scores was much flatter than previous lines. All of the data points fall within the 95% CI. Thus R3JU3 non-differentially incorporated the NCO treatment immediately. FB4U1 (a female blackbird) showed similar behaviour to the robin (Fig. 3.19). She non-differentially incorporated both of the novel colour treatments (NC – blue, NCO – green and almond) immediately. Both the NC and NCO treatment preference lines start and end inside the 95% CI. Both FB4U1 and R3JU3 escaped the training procedure, and so approached the test presentations as naïve predators. As naïve predators, they would have had no conception of a 'familiar' colour of bait. Another juvenile blackbird (B3JU, a blackbird) contacted the novel colour treatments (NC - blue, NCO – green and almond), but only ate them once during the short time it took part in the experiment (3 presentations).

3.8 Discussion 2:

Neither blackbirds nor robins were deterred by an unfamiliar odour of a familiar or unfamiliar coloured food, and both blackbirds and robins were slow to investigate a novel colour of a familiar food type. It is inappropriate to draw conclusions from the number of blackbirds (10) and robins (1) that incorporated the novel coloured treatments. The blackbirds and robins were competing for the same food resources, and naturally, the larger birds were. This unequal competition affected how long the two species spent at any
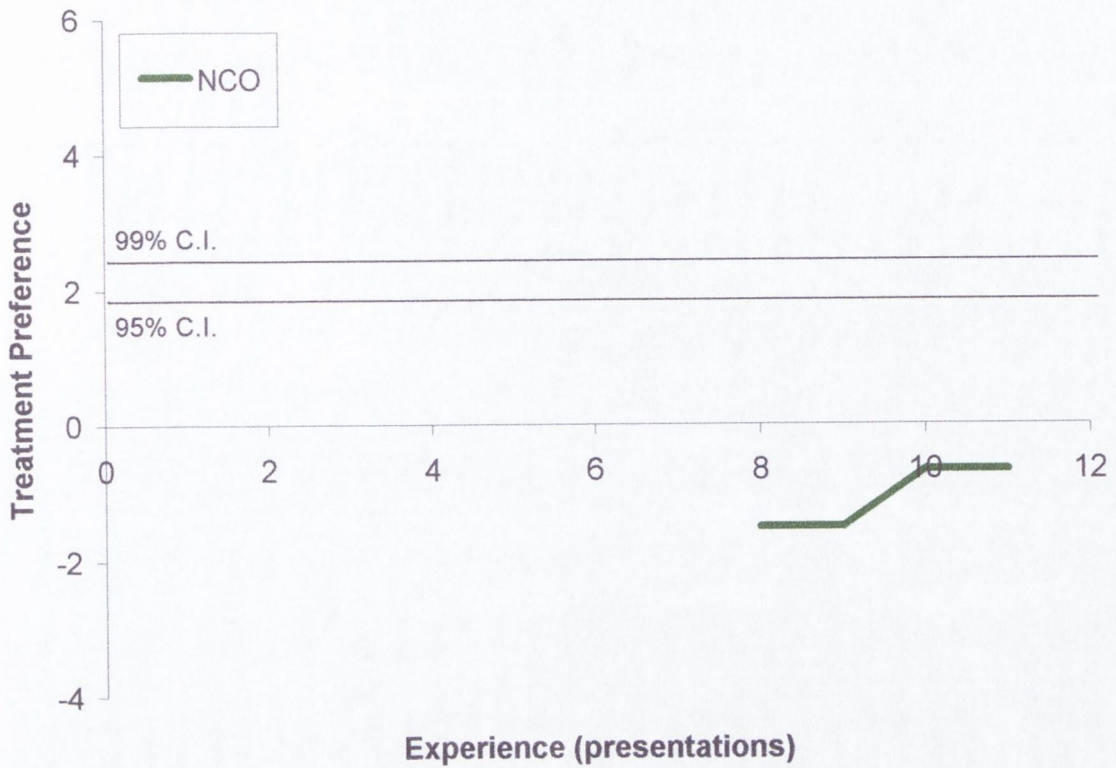
Figure 3.18. The dietary incorporation of a novel coloured food by a naive robin (R3JU3) over the 1999 field season. NCO (green line) represents novel green food in the presence of a novel almond odour, and is shown as a five point moving average. (For calculation of preference score see text.)
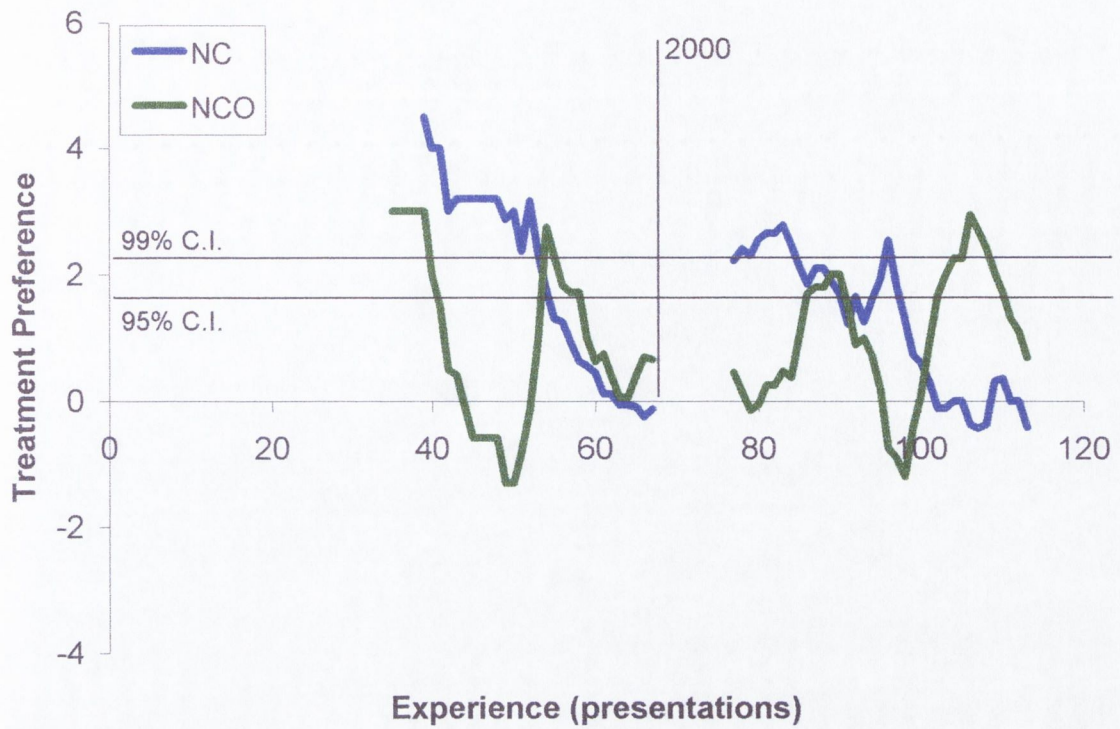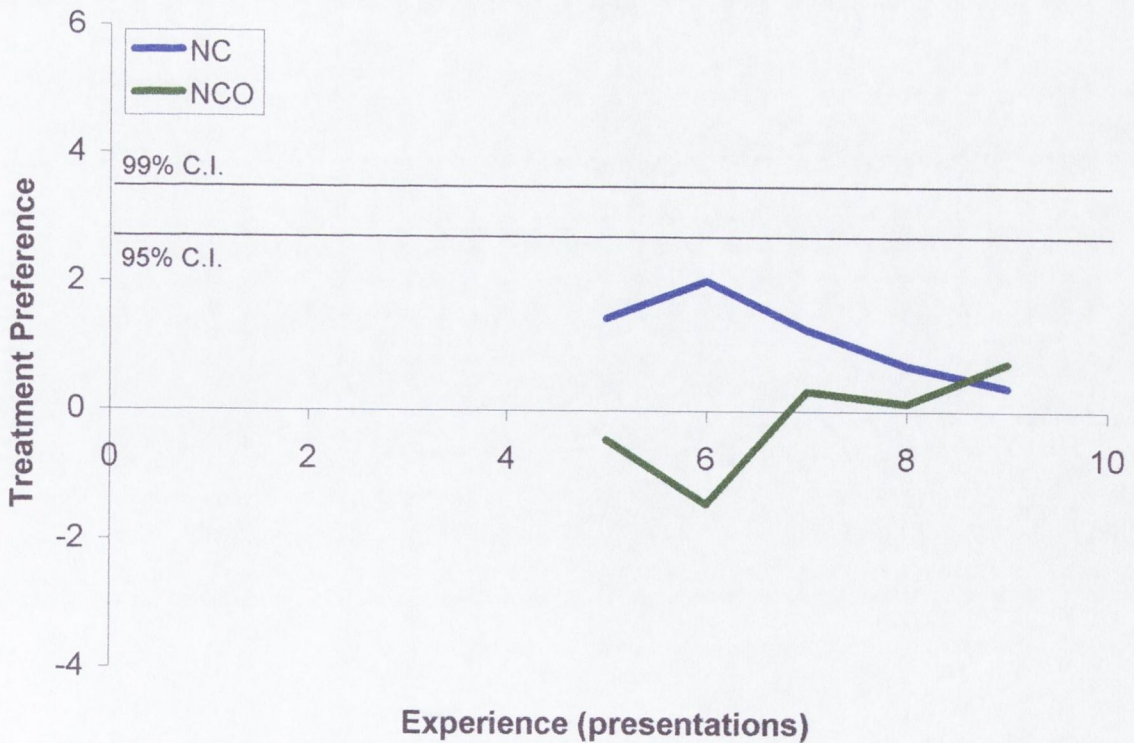

Figure 3.19. The dietary incorporation of novel coloured foods by a female blackbird (FB4U1) over the 2000 field season. NC (blue line) represents novel blue food, NCO (green line) represents novel green food in the presence of a novel almond odour. Both lines are shown as five point moving averages. (For calculation of preference score see text.)

feeding site. Many feeding bouts by robins took the form of 'raids', where they flew straight to a dish, took a single bait, and retreated to cover. In the 1999 and 2000 field seasons combined, robins only took all baits of familiar colour on 17% of presentations, while blackbirds took all baits of familiar colour on 88% of presentations. FB4U1 (a female blackbird) and R3JU3 (a juvenile robin) entered the experiment as naïve predators, and therefore provide useful comparisons with the rest of the trained birds, which had experience of the training food (FC). For green food (incorporated by both FB4U1 and R3JU3), the average time to contact (trained birds: mean = 48.0, n = 5; naïve birds: mean = 3.0, n = 2) and incorporation (trained birds: mean = 58.8, n = 5; naïve birds: mean = 4.5, n = 2) were lower for the naïve birds. As the naïve predators were not trained to recognise the uncoloured pastry as familiar, it might not be expected for them to leave other colours alone. This suggests that an insect might gain protection simply by having a novel colour morph, if it arose in a monomorphic population that was regularly predated. Such protection would not be afforded if the insect were considered an entirely new food type (as was presumably the case with the naïve predators). Of the three naïve birds, only one (R3JU3) was presented with red food. R3JU3 did not contact the red food, but did contact the blue food. Both FB4U1 and B3JU contacted the blue and green foods with which they were presented. This may be a sign of the importance of red as a warning signal of potential prey, but it might equally show red to be important to robins. As the red breast of the robin is known to be an important conspecific signal (Lack 1965; Mead 1984) it seems likely that it might affect other decisions involving red. As only one robin was considered here, further study is required before any firm conclusions may be drawn.

Preference of colours by the fully trained blackbirds did not differ among treatments. Of the birds which were presented the various colours: 41.4% (12/29) contacted blue, 45.5% (5/11) contacted red, and 50.0% (9/18) contacted green. Similarly 24.1% (7/29) incorporated blue, 27.3% (3/11) incorporated red, and 33.3% (6/18) incorporated green. From this summary it might appear that experienced blackbird predators are least afraid of novel green- and most afraid of novel blue-coloured morphs of familiar prey, but analysis of these data shows that the trends were not significant (contact: $\chi^2 = 0.34$, df = 2, p > 0.1, for incorporation: $\chi^2 = 0.47$, df = 2, p > 0.1). It might be predicted that red would have been least preferred, knowing its extensive use in warning signals (section 1.2). The preference for green in wild birds is interesting, as this is the colour of many cryptic insects. Previous experiments with chicks have shown a preference for green over

traditional warning colours in the presence of a 'warning' odour (Rowe & Guilford 1996). Experimenters have also found it difficult to make green treatments aversive to chicks (N. Marples pers. com.).

In contrast to the percentages of birds that contacted or incorporated each colour, a comparison of the average times taken to contact or incorporate them showed that for contact red (mean = 37.4, n = 5, s.e. = 18.0) > green (mean = 35.1, n = 9, s.e. = 11.3) > blue (mean = 29.7, n = 12, s.e. = 7.7). For incorporation green (mean = 50.8, n = 6, s.e. = 13.1) > blue (mean = 42.4, n = 7, s.e. = 7.8) > red (mean = 31.3, n = 3, s.e. = 16.2). These differences are non-significant (One-way ANOVA for contact: $F_{2, 23}$ = 0.13, p = 0.88, for incorporation: $F_{2, 13}$ = 0.54, p = 0.59). This does not consider birds that have had a great many presentations, and have failed to explore the novel colours. If all the blackbirds are included, by using MPV scores, we find a different result. This time, red (mean = 88.9, n = 11, s.e. = 30.4) > blue (mean = 67.4, n = 29, s.e. = 15.2) > green (mean = 48.1, n = 18, s.e. = 12.5) for contact, and red (mean = 113.1, n = 11, s.e. = 31.9) > blue (mean = 88.4, n = 29, s.e. = 15.4) > green (mean = 57.9, n = 18, s.e. = 11.9) for incorporation. This result considers all the birds that took part in the experiment, so should be a fairer reflection of the preferences of the population, but there was still no significant difference for selection of the colours (One-way ANOVA for contact: $F_{2, 55}$ = 0.95, p = 0.39, for incorporation: $F_{2, 55}$ = 1.75, p = 0.18).

Comparisons of the reactions of male and female blackbirds to respectively different treatments and colours of novel food are shown in figures 3.20 and 3.21. Apparent differences in the contact latencies of males and females to the NC and NCO treatments (Fig. 3.20), were not statistically different between the sexes (independent populations T-test for NC: t = -1.85, df = 27, p = 0.08; for NCO: t = -1.76, df = 27, p = 0.09 respectively). This may be because male birds, on average, received more presentations than female birds (Fig. 3.20). There are also apparent, but non-significant, differences between the latencies of males to contact food of the various novel colours (Fig. 3.21). However, there are no significant differences between the sexes to contact red (t = -1.09, df = 6.26, p = 0.31), blue (t = -1.59, df = 27, p = 0.12) or green food (t = -1.32, df = 16, p = 0.20).

One bird (MBGRL) was tested with three colours during the experiment. Having incorporated the two test colours it was decided to test it with a third. The results agree with previous work (Coppinger 1969; Coppinger 1970; Schlenoff 1984; Jones 1986; Mastrota & Mench 1995) that has shown experience reduces neophobia. Having taken 78
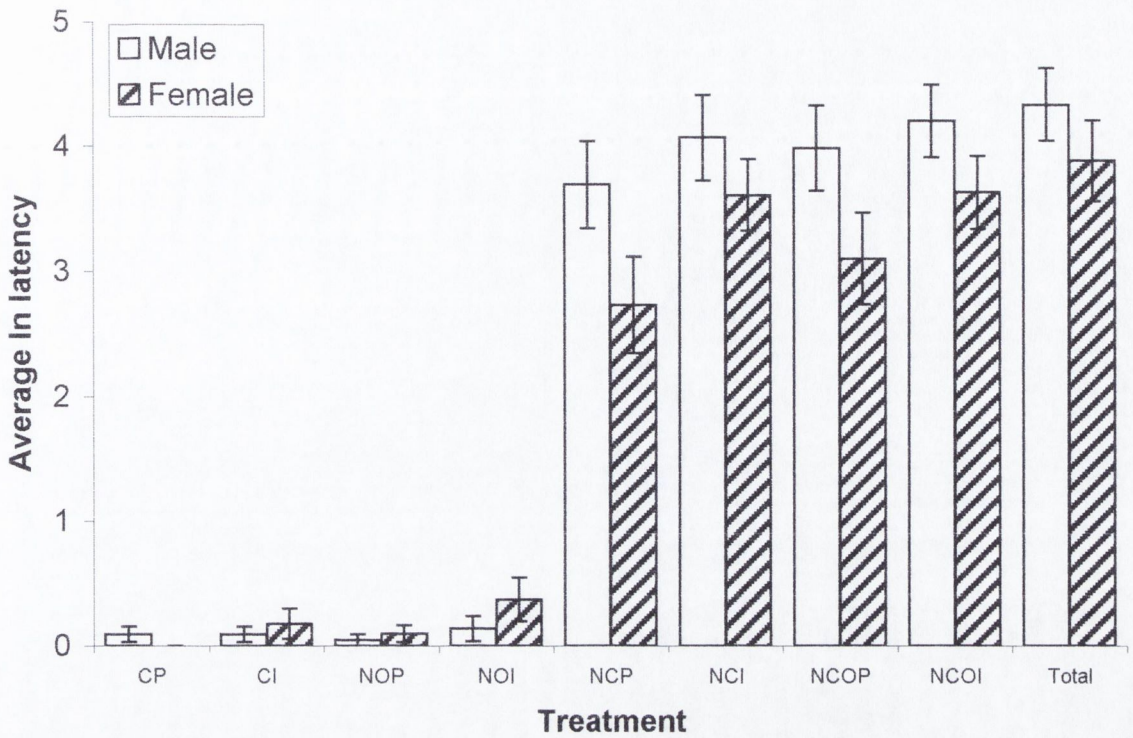
Figure 3.20. A comparison of the mean latencies of male (n = 15, empty columns) and female (n = 14, diagonally striped columns) blackbirds to contact (P) and incorporate (I) four bait treatments (C, NO, NC, NCO). Values shown are ln-transformed. Error bars are standard errors of the mean.
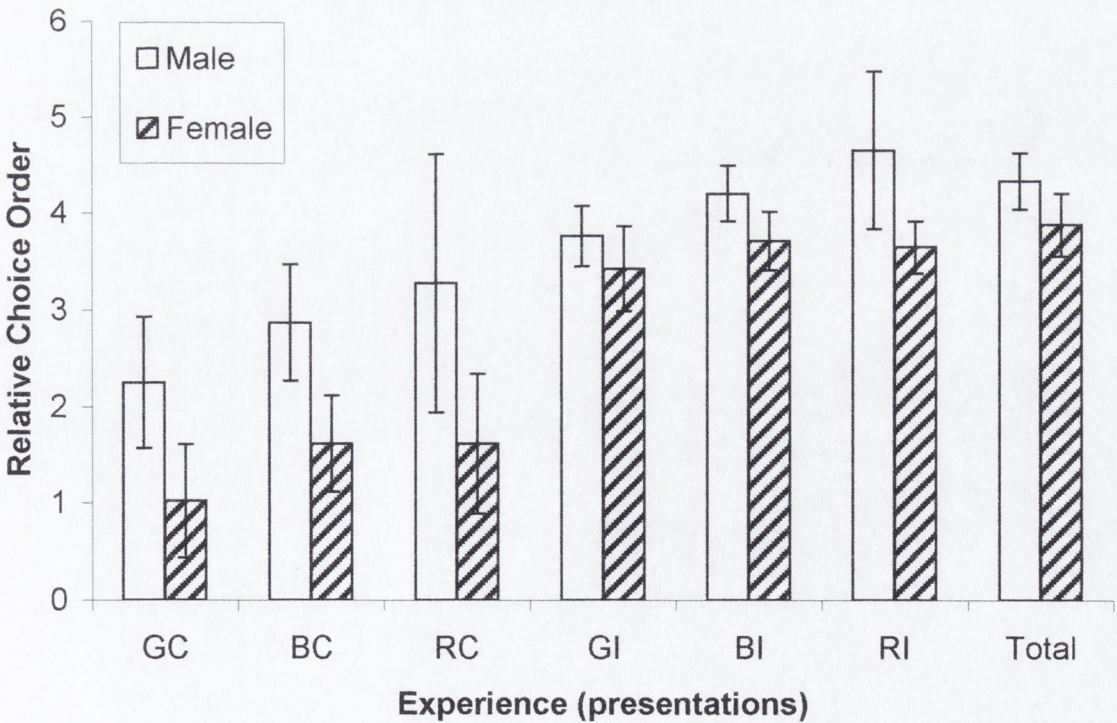
Figure 3.21. A comparison of the mean latencies of male (n = 15, empty columns) and female (n = 14, diagonally striped columns) blackbirds to contact (C) and incorporate (I) green (G), blue (B) and red (R) baits. Values shown are ln-transformed. Error bars are standard errors of the mean.

trials to contact both blue and green food, MBGRL took only 5 trials to contact the red food. DC was also reduced, as the red baits were also eaten on their fifth presentation (c.f. NC = blue eaten on 78th presentation). It is possible that MBGRL had reduced its DC towards coloured morphs of the pastry 'prey', and was therefore generalising this acceptance to novel food. Although, as all colours were eaten during these trials, this may simply support the view that dietary conservatism is reduced by previous experience. The birds that were tested across two field seasons provide further support of this idea. FB4U6 (Fig. 3.9), FBRRL (Fig. 3.10), MBRRL (Fig. 3.14), FB4U5 (Fig. 3.15), MB6U6 (Fig. 3.16) and FBRWR (Fig. 3.17) all showed reduced DC towards their incorporated coloured foods from the 1999 season to the 2000 season. Neophobia (i.e. time taken to contact) was similarly reduced.

It appears that the assessment process of the previously incorporated novel coloured treatments was also facilitated by experience. FB4U6 (Fig. 3.9), MB6U6 (Fig. 3.16) and FBRWR (Fig. 3.17) all non-differentially incorporated a coloured treatment immediately at the start of the 2000 field season, having previously non-differentially incorporated these in the 1999 season. Other birds showed a switch from non-differential incorporation at the end of the 1999 season to differential incorporation at the beginning of the 2000 season. The differential incorporation phases in these cases were however, shorter than the 1999 season (MBRRL, MB6U6 and FBRWR). So again, birds were showing an effect of experience on their incorporation of a novel coloured food. There was some suggestion that red is a more memorable colour than blue or green, as the two birds which had non-differentially incorporated red coloured food in the 1999 season (FB4U6 and MB6U6) maintained that incorporation level from the beginning of the 2000 season. Birds with similar experience of blue (MBRRL, FB4U5, MB6U6 and FBRWR) and green (MBRRL and FBRWR) failed to maintain non-differential incorporation at the start of the 2000 field season. It is of note that MB6U6 (Fig. 3.16), having non-differentially incorporated both red and blue baits by the end of the 1999 field season, only non-differentially incorporated the red baits at the start of the 2000 season. The sample sizes here are small, so firm conclusions cannot be drawn, but red is a classic signalling colour (Sillén-Tullberg 1985; Roper 1990) and may be more memorable as a result.

There was a strong indication that when a bird started to non-differentially incorporate a previously differentially incorporated food, this process interfered with the choices it made towards previously non-differentially incorporated foods. FB4U5 (Fig.

3.15), MB6U6 (Fig. 3.16) and FBRWR (Fig. 3.17) all demonstrated this process. It is possible that these birds were relegating their 'safe' coloured foods to a lower preference in order to focus on their assessment of the new coloured food. Such a strategy may allow a more thorough assessment of the new food.

3.9 <u>General Discussion:</u>

The discrepancy in the birds' responses to odour between this experiment and studies on laboratory held chicks (review in Roper 1999), where strong odour effects are found, may arise either because of species differences in olfactory bulb size (Bang & Cobb 1968; Healy & Guilford 1990), in differences between wild and laboratory conditions, or in differences of experience. Further work is needed to distinguish these factors, although the results from chapter 2 argue against bulb size being the main consideration. A potential criticism of the experimental procedure is that the odour from the NCO treatment may have been detectable from the other dishes, and therefore became familiar before the NCO treatment was eaten. This would effectively turn the NCO treatment into a second NC treatment. In order to address this concern, a second experiment was conducted in the laboratory using chicks (see Chapter 4).

There are indications that two birds (FBGBL – section 3.4 and MBMWR – section 3.7) were distinguishing between NC and NCO treatments at the time of incorporation. Both of these birds non-differentially incorporated the NC treatment, but only differentially incorporated the NCO treatments. Previous work has shown that blackbirds can be made to pay attention to cues that they normally ignore (Marples 1993). Perhaps odour is another cue that blackbirds perceive, but usually chose to ignore. Further studies in this area could help to resolve this question.

Using trends from the various incorporation graphs, it was possible to generate a 'typical' incorporation graph (Fig. 3.22). This graph shows that the incorporation of a novel food into the diet is a much more complex process than was previously thought (Marples *et al.* 1998). The process involves at least four stages:

1. Visual inspection of the novel food prior to contact.
2. Initial contact. The novel food may or may not be eaten.
3. Differential incorporation. The novel food is sampled occasionally and then regularly, but only when other more familiar food is not available.
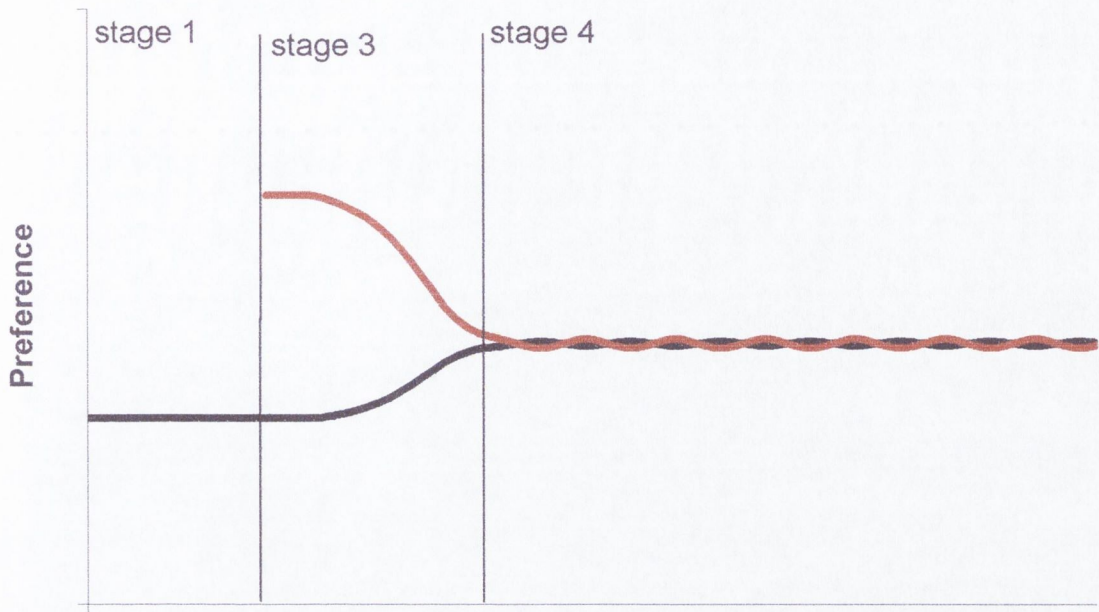
Experience

Figure 3.22. A generalised incorporation system for an experienced predator eating a familiar coloured prey morph (black line) and a novel coloured prey morph (red line), showing the various stages of incorporation. The preference scores relate to consumed prey only.

4.   Non-differential incorporation. The new food is eaten without preference for previously familiar food.

Stage 1 is illustrated in figure 3.22 as the position of the y-axis, since this is where the bird first encounters the NC prey. The start of the NC line is denoted as the start of stage 3. If first contact with the food was also first consumption of the food, then this point would mark the beginning of stage 2 too. However, stage 2 would then begin and end at this point. Stage 2 has not been indicated, as one needs to make assumptions about it in order to do so. Stage 4 is indicated where the FC and NC lines come into contact. As experimental birds have illustrated, there is some stochastic variation in food selection at stage 4, so rather than show the two lines as superimposed, they have been shown as intertwined (see Fig. 3.9). The duration of stages 1 and 2 combined can be deduced by the time interval between the start of the FC line and the NC line. Stage 2 cannot be placed with any degree of certainty, unless assumptions are made about the nature of the contact the predator has with the prey. From the blackbird data for the 1999/2000 season (see appendix 3) only 23% of birds (7 out of 30) distinguished stage 2 from stage 3, i.e. their first contact with a bait did not involve eating it. Although most birds did eat the novel treatment at first contact, others were more cautious. FBRRL (a female blackbird) made contact with the NC treatment (blue) on 16 occasions, but never ate it.

Stage 1 may be considered by some to be similar to neophobia, but the birds need not show 'fear' of the food or avoidance of it, they simply refuse to eat it. It is better to say that this stage includes neophobia. This stage may last a very long time. Three blackbirds (MB5U6, MBBYL & MBGL) failed to make contact with either coloured treatment after more than 200 presentations. Considering the transition from inspection of the novel food to contact with the novel food as one stage may well be an oversimplification. Similarly, once the NC food has been non-differentially incorporated into the diet, further complexities may still be present. We have already seen that some birds treat a non-differentially incorporated food of novel colour differently from FC food when they incorporate a second food of novel colour (section 3.7). Thus, a memory of discrimination persists for an unknown duration.

These four stages show that dietary conservatism is more complex than originally suggested by Marples et al. (1998). Knowing that some birds have been demonstrated to show a genetic aspect to their latency to contact novel food (Marples & Brakefield 1995), it

might be predicted that birds that are slow to progress from the second stage to the third stage may also be slow to progress from the third stage to the fourth stage. My data on the birds that have incorporated these foods to date does not support this conjecture (see appendix 3). It appears there may be different processes governing the different stages of dietary incorporation.

The finding that dietary incorporation is a multi-staged process has important implications for our understanding of the evolution of aposematism. Unlike the accepted doctrine that conspicuously coloured prey suffer increased predation (Gittleman *et al.* 1980; Lindström *et al.* 1999b), the added protection inherent in this four-stage process reduces attacks on novel-coloured morphs to a minimum. If familiar alternatives were available in abundance, as would be likely for a newly evolved morph, there appears little likelihood that experienced predators would attack novel-coloured prey. Even after the fourth stage of incorporation the newer food still maintained some level of protection from the less hungry birds. The reluctance of individual birds to sample novel morphs has been demonstrated to last longer than two years. This could provide protection of a novel morph for several generations of an invertebrate prey species. Such protection might enable a small population to survive and develop chemical defence, and so become a separate, viable, aposematically defended population. Such an evolutionary pathway has been previously discounted (Guilford 1990). This system depends on the predators having experience with the established population. It therefore appears important to consider the relative abundance of experienced and naïve predators in any given ecosystem to calculate their impact on the appearance of a new aposematic prey animal. Similarly, it seems important to consider the role that parents may play in the food preferences of altricial young (Avery 1996). Upon fledging, altricial birds may not be genuinely naïve predators. However, the small data set gathered on juvenile birds (B3JU & R3JU3) indicates that if they had been fed on FC baits, this had not provided them with a search image of a specific colour. They were to all intents and purposes entering the trial as naïve predators.

The birds under test here were in a public park, where they appeared to encounter a relatively high number of novel foods from picnickers. It may well be that these birds were actually less conservative in their diets than other more rural birds, although the results given here show my birds to be more conservative than others from a previous study on a university campus (Marples *et al.* 1998).

3.10 Conclusions:

Wild blackbirds do not react to novel foods in the same way that laboratory-held chicks
do. Despite some indication that blackbirds and chicks can detect odours of the same intensity, it appears that blackbirds do not generally use odour information to make decisions about the safety of novel foods. The process by which a blackbird incorporates a novel food into its diet is a complex one, and this has suggested a number of more general conclusions:

- The incorporation process of a novel food is a multi-staged process.
- This process contains at least four distinct stages.
- The process of dietary incorporation is influenced by both the neophobic and DC responses of the predator.
- Wild birds show a wide variation in their individual responses to novel colour morphs of a known prey type.
- A novel colour prey morph may derive protection from its colour alone.
- Naïve predators are not deterred by novel colour morphs of prey.
- Both neophobia and DC appear to be reduced by previous experience.
- Memory of incorporation varies with the level of experience.
- Memory for incorporation may be affected by the prey colour.
- Odour does not appear to be important in the food choices of robins and blackbirds.

Chapter 4

The responses of laboratory-held chicks to novel foods with combinations of novel colour and novel odour

4.1 <u>Introduction:</u>

Following concerns about the interaction of odours in the solitary wild bird experiment (section 3.8), the experimental set-up was tested in the laboratory. Chicks were chosen as the predator for these trials, as they have been shown to use odour cues in choices about new foods (Marples & Roper 1996). Neophobic responses of chicks have been shown to be much shorter than wild blackbirds (Marples & Kelly 1999), so these trials allowed a quick assessment of chicks' responses to the simultaneous presentation design, and distinguished species-specific reactions from general ones.

4.2 <u>Materials and Methods:</u>

The presentation dishes, pastry and treatment notation used for this experiment were as described in section 3.6. The bait size was decreased to 0.5cm x 0.5cm x approx. 0.15cm to reflect the smaller size of the chicks. Bait density was maintained at the previous level (section 3.2) to avoid any unwanted frequency-dependent effects (see section 1.3). Day-old male chicks of the Cobb 500 strain were obtained from a commercial supplier. They were individually colour marked on their heads. During training the chicks were familiarised with the Control treatment (C). Outside of training they were allowed food (chick starter crumbs) and water *ad libitum*.

The chicks underwent a two-step training procedure over two days prior to testing. The first step familiarised them with the edibility and colour contrast of the training food (white pastry – see section 3.2). After a one-hour food deprivation period, groups of chicks (eight or nine) were placed in a test arena (a high-walled white card cylinder of cross-section 60cm) and presented with small triangles of pastry (surface area: 0.5cm x 0.5cm x 0.71cm) on a circular piece of black card (9cm diameter). Triangles were chosen as they had previously been shown to be the shape of food that chicks found least neophobic (L. Harris unpublished data). Once the birds were readily eating the triangles, they were presented with the small squares of pastry (surface area: 0.5cm x 0.5cm – C treatment). This 'pre-training' session lasted no more than 20 minutes. The following day, the chicks were deprived of food for one hour, and tested individually in the enclosure with a control dish and the C treatment. As chicks are gregarious, and known to become distressed in isolation, these 'training' trials were conducted in a divided test arena. On one side was a buddy chick (a chick not used for testing) with a supply of the training food, and on the

other was the chick to be trained. A sheet of chicken mesh (mesh size = 13mm) separated the chicks. The baits were presented two at a time in the presentation dish to simulate the testing conditions. Having completed the pre-training and training procedures, the chicks were assumed to be familiar with the test arena, the training food and the presentation dishes.

The test chicks were divided into two groups at random, with 31 birds in each group. Group 1 was assigned: NO = vanilla, NC = blue, NCO = green + almond. This arrangement had been presented to 18 blackbirds (section 3.6). Group 2 was assigned: NO = vanilla, NC = green, NCO = blue + almond. On day three the testing began. Test chicks were placed in the arena with a full selection of the four treatments (two baits in each dish). Dishes were aligned and rotated as described in section 3.2. The chicks were given time to investigate all the dishes before they were considered to have completed the presentation. This allowed them a maximum of 60 seconds for each presentation. Each bird received two or three such presentations per day.

4.3 <u>Data Analysis:</u>

The latency data was not normally distributed (Shapiro-Wilk p values < 0.01), so analysis was performed using non-parametric tests. Statistical tests were applied with SPSS© v10.0.5 (SPSS Inc. 1989 - 1999).

4.4 <u>Results:</u>

Data from groups 1 and 2 are shown as latencies to contact (Fig. 4.1) and incorporate (Fig. 4.2) the various treatments (see appendix 4). Minimum possible values for incorporation (MPVs – see section 2.4) were used to expand the data sets. When considering the birds from groups 1 and 2 together, there were significant differences between the various treatments (contact: Friedman χ^2 = 13.7, df = 3, p = 0.003, incorporation: Friedman χ^2 = 47.0, df = 3, p < 0.001). These differences are detailed in table 4.1 (contact latencies) and table 4.2 (incorporation latencies). Table 4.1 shows that there was no significant difference between the contact times of the C and NO treatments. This shows that there was no main effect of odour for contacting a novel food. Table 4.1 also shows that there was no significant difference between the contact times of the NC and NCO treatments. This shows there was no interaction between the novel odour and colour cues when the chicks contacted a novel food. A general comparison of the coloured

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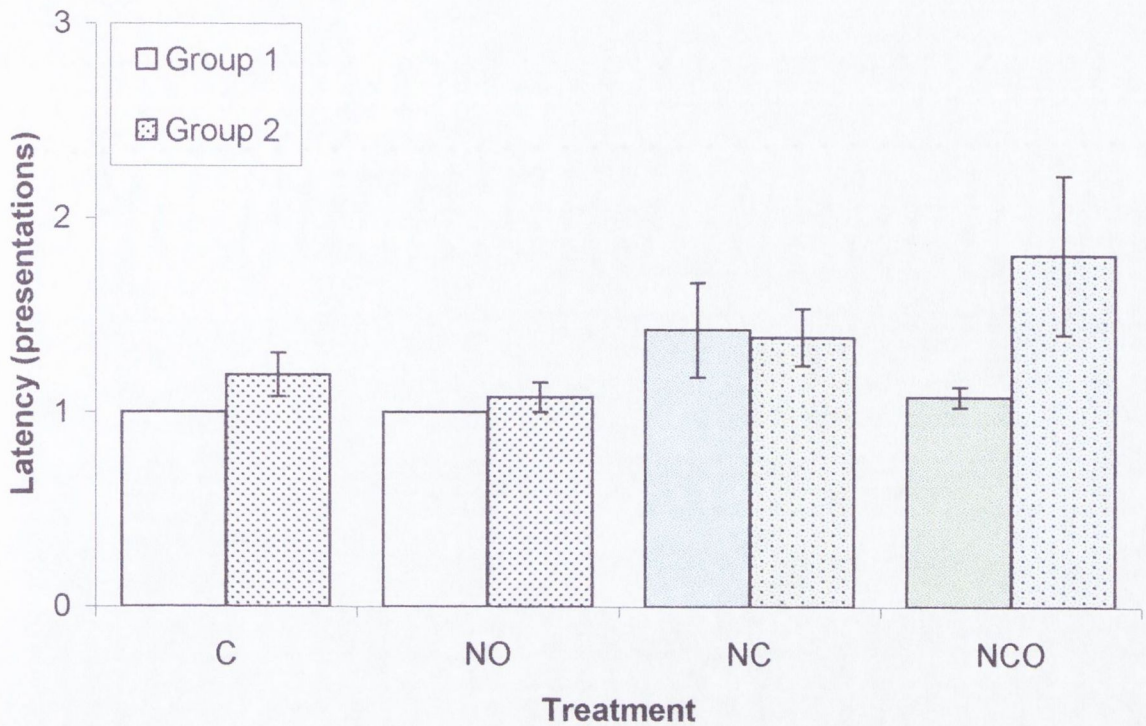
Figure 4.1. Comparative latencies of chicks to contact three novel treatments of pastry including MPV scores (see text). Data are separated into experimental groups 1 and 2 (n=26 in both cases) to compare individual colour effects. Standard errors of the mean are shown when they are significantly greater than zero.
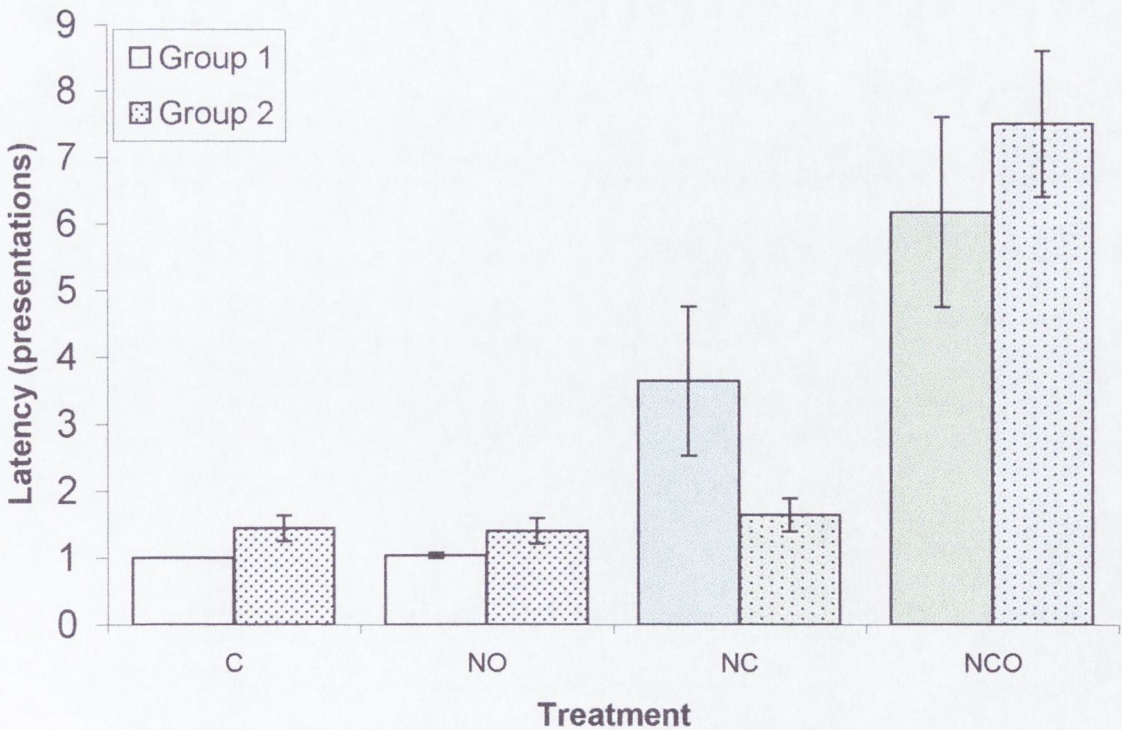
Figure 4.2. Comparative latencies of chicks to incorporate three novel treatments of pastry into their diet, including MPV scores (see text). Data are separated into experimental groups 1 and 2 (n=26 in both cases) to compare individual colour effects. Standard errors of the mean are shown when they are significantly greater than zero.

treatments with the uncoloured treatments showed the chicks were distinguishing between them. So there was a main effect of colour for contacting novel food. Table 4.2 shows that

	Control	Novel Odour	Novel Colour
Novel Odour	Z = -1.3 p = 0.18		
Novel Colour	Z = -2.2 p = 0.03	Z = -2.5 p = 0.01	
Doubly Novel	Z = -1.9 p = 0.06	Z = -2.6 p = 0.01	Z = -0.5 p = 0.65

Table 4.1. Comparison of contact latencies for the four treatments (groups 1 and 2 combined) using Wilcoxon matched pairs tests.

there was no significant difference between the contact times of the C and NO treatments. This shows that there was no main effect of odour for incorporating a novel food. Table 4.2 also shows that there was a significant difference between the contact times of the NC and NCO treatments. This shows there was an interaction between the novel odour and colour cues when the chicks contacted a novel food. A general comparison of the coloured treatments with the uncoloured treatments showed the chicks were distinguishing between them. So there was a main effect of colour for contacting novel food.

	Control	Novel Odour	Novel Colour
Novel Odour	Z = 0.0 p = 1.0		
Novel Colour	Z = -2.8 p = 0.004	Z = -3.0 p = 0.003	
Doubly Novel	Z = -3.9 p < 0.001	Z = -4.0 p < 0.001	Z = -3.3 p = 0.001

Table 4.2. Comparison of incorporation latencies for the four treatments (groups 1 and 2 combined) using Wilcoxon matched pairs tests.

In summary, there was a main effect of novel colour for both contact and incorporation of a familiar food, but there was no main effect of novel odour for either

contact or incorporation. There was an interaction of novel colour and odour for incorporation of a familiar food, but no interaction for contact. This suggests that there are different processes involved with contact and incorporation.

Table 4.3 and table 4.4 show the incorporation latencies of the chicks within the two experimental groups. Table 4.3 shows that group 1 birds displayed no main effect of odour

	Control	Novel Odour	Novel Colour
Novel Odour	Z = -1.0 p = 0.32		
Novel Colour	Z = -2.4 p = 0.02	Z = -2.4 p = 0.02	
Doubly Novel	Z = -2.7 p = 0.007	Z = -2.7 p = 0.007	Z = -1.6 p = 0.11

Table 4.3. Comparison of incorporation latencies for the four treatments in group 1 using Wilcoxon matched pairs tests.

in their incorporation latencies (C vs NO), but a main effect of colour (significance between coloured and uncoloured treatments). These results agree with the grouped analyses (Table 4.2). Group 1 did not show an interaction between novel colour and odour (NC vs NCO).

	Control	Novel Odour	Novel Colour
Novel Odour	Z = -1.0 p = 0.32		
Novel Colour	Z = -1.7 p = 0.08	Z = -1.9 p = 0.06	
Doubly Novel	Z = -3.0 p = 0.003	Z = -3.0 p = 0.003	Z = -3.0 p = 0.003

Table 4.4. Comparison of incorporation latencies for the four treatments in group 2 using Wilcoxon matched pairs tests.

Group 2 (Table 4.4) showed the same independent effect of odour, but not of colour (note C vs NC). Group 2 also showed an interaction of novel colour and odour (NC vs NCO). It is apparent that the chicks in the different groups were reacting differently to the treatments. Group 1 used blue as NC, and green and almond as NCO, whereas Group 2 used green as NC and blue and almond as NCO. The chicks must have been reacting differently to the different colours, as the odours were the same across groups. There was no significant difference between the incorporation latencies for the C and NC (green) treatments in group 2 (Table 4.4). When NC was blue, (group 1) this difference was evident (Table 4.3). Conversely, group 1 (Table 4.3) birds failed to show an interaction between novel colour and odour (NC vs NCO treatments), whereas group 2 birds (Table 4.4) did show this interaction. Group 1 birds failed to differentiate between novel blue food, and novel green food with a novel almond odour. Group 2 birds did differentiate between novel green food, and novel blue food with a novel odour.

4.5 Discussion:

The results obtained for the NO treatment indicate that novel almond or vanilla cues on their own are not aversive. This supports the work of Marples and Roper (1996), although Jones (1987) has shown that the odour of orange oil can be aversive. The addition of a novel almond odour was shown to increase the latency of chicks to eat a novel blue-or green-coloured food (Figure 4.2).

The original question this experiment was designed to answer was whether the two odours in the presentation dishes would be considered as familiar when the birds came to eat the food in them. As has been demonstrated, there was a strong interaction between colour and odour, so the odour in the NCO treatment was not treated as familiar. As chicks reacted differently to the NC and NCO treatments, it is clear that blackbirds and chicks behave differently to these cues (section 3.9). Because the odour component of the NCO treatment was not treated as familiar, this indicates that the odour intensity was an important aspect of the signal. It seems inconceivable that the chicks would not have registered the almond odour (the odour in the NCO treatment for both groups 1 and 2) before contacting the NCO treatment, as both odours permeated the experimental arena and were obvious to human observers. However, the relative intensities of the odours would change as the chicks moved from one presentation dish to another, being most intense at

the dishes where the olfactants were applied. It is reasonable to assume then, that the chicks were using the intensity of the odours to determine their source. As a result, the chicks did not treat a familiar odour as unimportant. This finding does not agree with Rowe and Guilford (1999). They showed that pyrazine odour needed to be novel in order to enhance discrimination between green and yellow food. Such differences may have been owing to the comparative nature of Rowe and Guilford's study or the reaction of chicks towards green food (see below).

The interaction between novel colour and novel odour appeared to make the chicks more wary about eating food (i.e. incorporation of a treatment), but seemed relatively unimportant if they were just going to make contact with it. Work by Martareche and co-workers (2000) has demonstrated that chicks use pecking as a means of examining food, and not simply for eating. Contact with a food should be considered to be a less important commitment on the part of the chick than eating a food. As the results demonstrated, when there was a colour component to the novel food, the latency to contact (Figure 4.1) it was significantly different from the latency to eat it (Figure 4.2). When a novel odour was presented with a novel colour there was a synergistic effect of the two novel stimuli. These results support those of Marples and Roper (1996), although they presented the treatments individually.

The experiment here has not considered the possibility that almond may be a more aversive odour than vanilla, and may therefore be more aversive in combination with a novel colour. Previous studies have shown that both vanilla and almond odours can overshadow novel colour cues (Marples & Roper 1996), so may be equally aversive. This problem could be directly addressed by a second experiment of a similar design, but using vanilla in the NCO treatment and almond in the NO treatment.

A consideration of the colour of the novel treatment, rather than simply its novelty, appears to show a distinction between green and blue. Was green sending a 'friendly' signal to the chicks? Neither green as NC or NCO was contacted significantly later than the C treatment, but blue was. Similarly, there was no difference between the latency to eat green food as NC and C (group 2). When NC was blue; there was a significant difference. Only when green was the NCO treatment was it eaten significantly later than the C treatment. The comparison between blue as NC and green as NCO (Table 4.3) was not significant. This suggests that either blue is more, or green is less 'frightening', as a novel colour. These two colours are generally considered to be neutral (Roper & Marples 1997a,

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b). There is no strong evidence to suggest blue as a classic warning colour, although Wrazidlo (1986) found that young pheasants (*Phasianus colchicus*) were more averse to blue than green food. Perhaps green is an innately preferred colour. Do other birds find green 'friendly'? Looking at the results from the blackbird experiment (section 3.7), it appears that they do. Do birds have an innate preference for green coloured food? If so, a classically cryptic insect (green) would need to develop particularly powerful defences to discourage a predator from attacking it. If the cryptic insect developed a different colour morph though, this would appear to offer protection, at least in the short term.

If neophobia is the latency to approach and DC is the latency to incorporate, then we can see the two processes at work in figures 4.1 and 4.2. The contact latency can be considered to be the latency owing to the neophobic aspect of the colour, as once chicks have approached a food they use pecks to investigate it further (Martaresche *et al.* 2000). The differences between the contact and incorporation latencies are the times from first sampling of the food to full acceptance, although it was not possible to test the chicks for non-differential incorporation here (see section 3.4), due to the brief presentation histories of the birds. So the differences between the contact and incorporation latencies are effectively the latencies due to the DC aspects of the birds' behaviour. If we look at figures 4.1 and 4.2 in this new light, we can see that DC was the important process when the food had a novel colour. The variation in contact times (relating to neophobia) for familiar and novel coloured foods was small, whereas the same variation in incorporation times (relating to DC) was much larger. Such a claim is supported by the work of Jones (1986). He showed that reducing neophobia in chicks did not reduce significantly their latency to eat food of a novel colour, where reduction in DC did. Jones demonstrated that chicks reduced their activities associated with fear if their home cages underwent environmental enrichment, but this environmental enrichment failed to produce a significant reduction in their latency to eat novel blue food. Experience of eating several novel coloured foods, however, did reduce the latency of the chicks to eat novel blue food. Eating novel coloured foods was effectively reducing the DC of the chicks. This finding is consistent with the latency to eat novel coloured food being largely composed of a DC component.

The birds used in this experiment were chicks, and a number of problems can be raised against their suitability as general models (section 1.7). Blackbirds appeared to show both a neophobic and a DC effect of colour (section 3.7). Whereas chicks showed very small changes in neophobia for contacting novel food, blackbirds showed dramatic

differences. This was probably, at least in part, owing to behavioural differences between these species. Nonetheless, an important point remains. If there were only small differences between the neophobic effects of the chicks for the four food treatments, then the differences found must have been owing primarily to DC.

4.6 <u>Conclusions:</u>

Chicks and blackbirds differ in their responses to combinations of novel colours and novel odours in familiar foods. Further important observations about the feeding behaviour of chicks include:

- Novelty of an odour cue is not essential to produce a synergistic avoidance effect with a novel colour.
- The intensity of an odour appears to be an important indicator of its source.
- The latency of a chick to eat a novel coloured food appears to owe only a small part to neophobia.
- Differences in latency to eat familiar and novel coloured foods appear to be due, in the main, to dietary conservatism.
- Green is preferred over blue as a food colour by chicks.
- Birds may innately prefer green food.

Chapter 5

The responses of adult social wild passerines to novel foods with combinations of novel colour and novel odour

5.1 Introduction:

Social species have been demonstrated to exhibit lower levels of neophobia in a flock (section 1.6). It appears that this may also be true of dietary conservatism (Pettersson 1959; Fryday & Grieg-Smith 1994). Novel colours of familiar prey have been demonstrated to deter predation in solitary wild birds (chapter 3). The finding that wild solitary passerines demonstrate significant neophobic and DC effects when presented with familiar food of a novel colour suggests that social passerine species will have to overcome both of these effects in order to incorporate foods with the same novel cues. If social birds were found to reject familiar food of a novel colour, in favour of the familiar colour, this would add weight to the argument that the development of a novel colour morph is a potential first step towards the evolution of an aposematic species (chapter 3).

A captive social granivorous species (the zebra finch) has been demonstrated to use odour in its assessment of food of a novel colour (chapter 2). As both greenfinches and chaffinches are social granivores (at least in the winter), odour cues are likely to be important to them too. Birds are a pest to stored grain (Kelly 1996), and are required to be controlled under international standard ISO 6322-3. In order to protect grain from granivorous pests, it is often necessary to treat the grain in some way. Treatment may be the application of a coloured dye (Pawlina & Proulx 1996) or an unpalatable agent (Avery *et al.* 1995; Watkins *et al.* 1995; Avery *et al.* 1997), or both (Avery 1984). This trial assessed the possibility of using indirect protection for the stored grain. By using incident light and odours, the grain itself was neither physically nor chemically altered. In the past, protection of grain crops has relied on exploiting avian neophobia and aversion learning. A better understanding of basic avian feeding behaviour may have similar applications.

5.2 Materials and Methods:

An experimental area was chosen where social species were known to congregate during the winter months (arable farmland in the Wexford Slobs). Two feeding sites were chosen within this area, where birds were familiar with wheat grain as a food source. There were at least 20 individuals regularly feeding at these sites. The main species eating the grain were greenfinch, chaffinch and great tit.

Four modified, black plastic household dustbins (36cm diameter at base, 65cm height) were used to present the grain to the birds (Fig. 5.1). The grain lay in a thin layer (~1cm) on the bottom of a small, white plastic bucket (25cm diameter, 21cm height) inside these bins. A small channel (made from 25mm internal diameter plastic tubing) on the inner surface of the bucket was used to present the olfactants. This channel was filled with filter paper, which was subsequently soaked with the olfactant. This technique prevented spillage, and therefore direct contamination of the grain. Presentations requiring no olfactant used water-soaked filter paper. The lids of the dustbins were partially replaced with theatrical lighting filters to give the impression of coloured grain, without spoiling the grain for future use. The colours used for this trial were primary red and primary green. Presentations requiring no coloration used translucent white plastic in place of the filters. These three different colour treatments produced roughly equal light intensities within the bins (200 lux) when measured with a light meter placed on the base of the grain bucket.

Under test conditions access to a bin was through a small hole in its side (5cm diameter, 48cm from ground level). A piece of sturdy dowel (0.8cm diameter) ran through the bin just below the access hole, allowing perching adjacent to the hole on entry and exit. Birds were initially trained to use the bins by leaving the lids off. When accustomed to feeding from the bins, white control lids were placed on the bins and a small feeder was placed adjacent to the access hole. Once birds were regularly entering the bins, the feeder was removed and testing began.

At testing time, the birds were presented with four treatments in four neighbouring bins: Control (C - olfactant = water, filter = white), food of novel odour (NO - olfactant = vanilla, filter = white), food of novel colour (NC - olfactant = water, filter = red), and doubly novel food (NCO - olfactant = almond, filter = green). The colour and odour used in the NCO treatment were different from the NO and NC treatments, in order to maintain the novelty of these stimuli.

The four bins were placed in a diamond arrangement on the cardinal points. Access holes faced diagonally out from the four corners of this diamond. The bins were rotated by one position daily, to control against bias owing to weather and access from neighbouring perches. 'Electronic eye' sensors were designed and hand-built prior to the start of the field trials. Completed sensors were placed just behind the entrance/exit holes to monitor the number of individuals visiting each bin. Counts were taken daily. The weight of grain within each bin was also monitored daily.
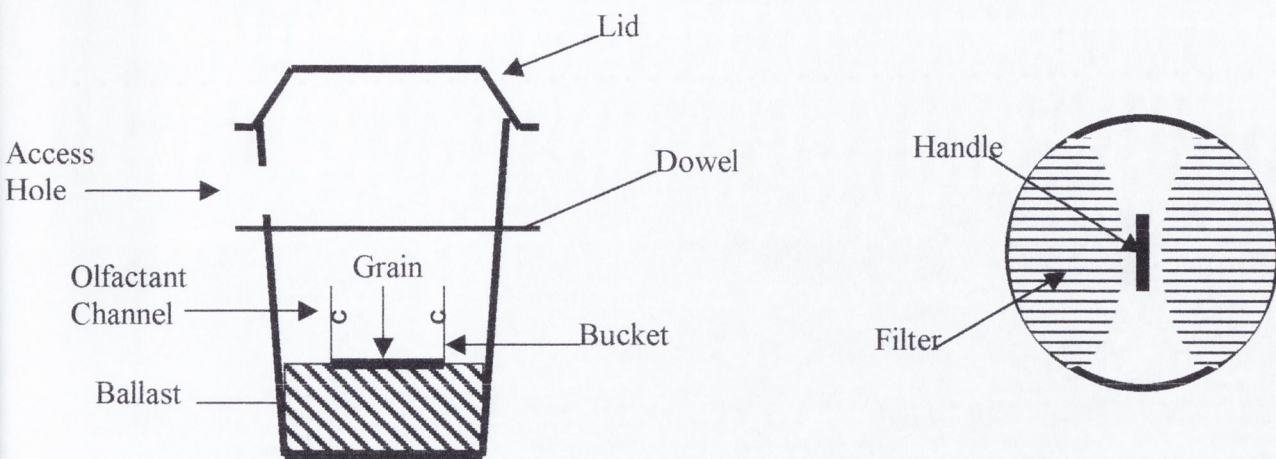
Figure 5.1. Cross-section through a presentation bin with surface elevation of the lid.

5.3 Data Analysis:

Statistical tests were performed on SPSS© v10.0.5 (SPSS Inc. 1989 - 1999). Analysis of a combined dataset of all weight changes showed the data to be normally distributed.

5.4 Results:

During the study period, the temperatures frequently dropped below zero overnight. These cold temperatures had detrimental effects on the counters and the electronic eye systems. Battery life was dramatically reduced, and the counters varied from unresponsive to hyperactive. The results must therefore rely solely on the weight data gathered for the two sites (Fig. 5.2 and appendix 5). Values plotted for mean weight change are the mean cumulative changes for the treatments from the two sites. Unfortunately, it was only possible to run the experiment for 10 days, owing to time restrictions.

Initial viewing of the data shown in figure 5.2 reveals an unusual phenomenon. Although the graph is supposed to plot grain consumption, some of the treatments gained weight (generated negative grain consumption values) during the experiment. This gain was caused by rainwater entering the bins, as well as some absorption of moisture by the dried grain. It would appear that there was no consumption of grain from the NCO bins, and the grain absorbed the rainwater that entered the bin. This produced a net increase in weight of the NCO treatment over time. The NC mean grain consumption rises to 5g by day 6, but falls back to –2.5g by the end of the experiment. It appears that some food was eaten from this treatment, but rainwater entered one of the NC bins on day 10. Rainwater 'contamination' occurred for the C (day 7) and NO (day 8) treatments too.

If we can ignore these 'blips' in the consumption rates for the treatments, we see that both the C and NO treatments show steady but different rates of weight change. In order to analyse these rates, a univariate ANOVA was chosen. Such an analysis is useful for time series data. The analysis used cumulative mean grain consumption as the dependent variable, colour and odour as fixed factors, and time (days) as a covariate. It therefore effectively compared the rates of consumption for the four treatments. This analysis gave a main effect for both odour ($F_{1, 39} = 40.0$, $p < 0.001$) and colour ($F_{1, 39} = 116.1$, $p < 0.001$), but no interaction between colour and odour ($F_{1, 39} = 0.13$, $p = 0.72$).
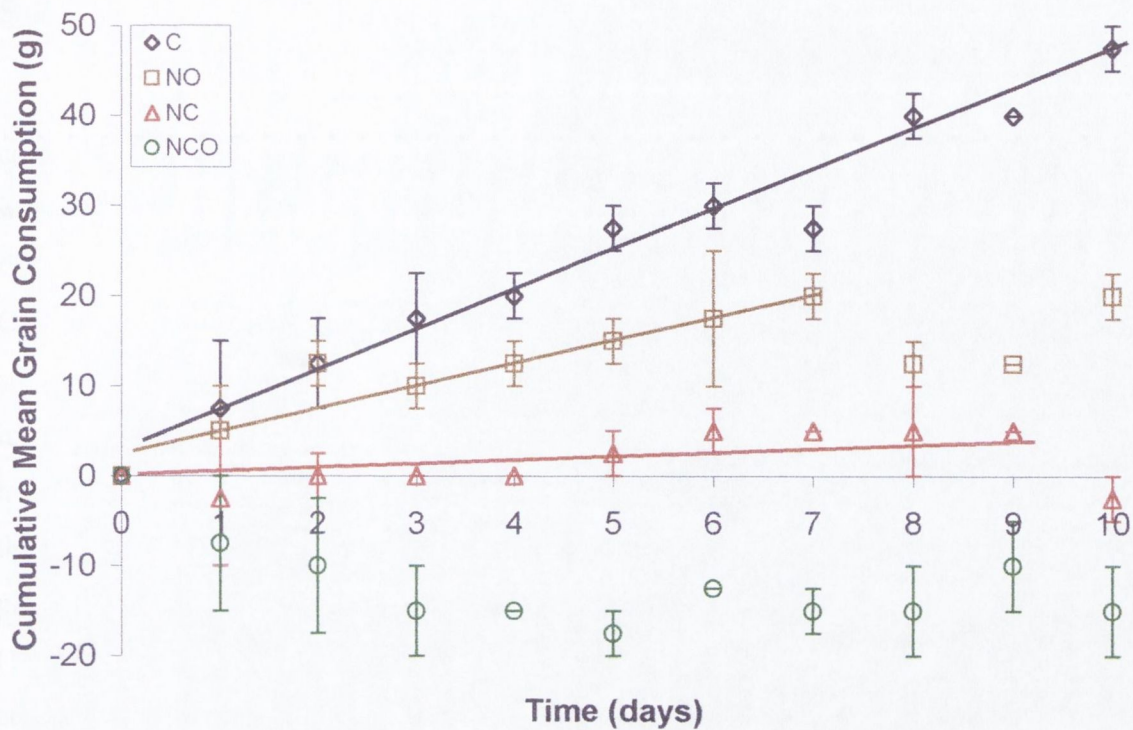
Figure 5.2. Cumulative mean consumption of grain by two social wild bird flocks over an eleven day period. All points show the standard error of the mean.

5.5 **Discussion:**

The main effect of colour demonstrates that these wild birds were sensitive to colour changes of their food. This idea is not unusual, as several authors have shown that wild or wild-caught passerines have a preference for familiar coloured food (Ridsdale & Granett 1969; Brunner & Coman 1983; Pawlina & Proulx 1996; Marples *et al.* 1998). Chapters 2, 3 and 4 also demonstrate that birds prefer familiar coloured food. All of these studies had coloured the food used, and it is possible in some cases that this may have inadvertently altered the test food to make it multimodally defended (i.e. colouring may have produced unpalatability or mild toxicity, as well as colour). This study demonstrates that coloured incident light on uncoloured food can produce the same avoidance effects as physically coloured food, and may be the first study to do so. A study by Osorio *et al.* (1999) also avoided directly colouring the food. They trained chicks to feed from orange-coloured paper parcels containing chick crumbs. These birds were then tested with a variety of slightly different colours, and found to prefer the original at least three times more than the alternatives. Although the different ink colours used might have produced different flavours or odours to the paper parcels, it is likely that such differences were very small.

The avoidance of the novel-coloured food is difficult to categorise. During previous studies (chapters 2, 3 & 4) the birds were observed throughout the food presentation, and so approach, contact and consumption of the food had been possible to differentiate. The presentation system used in the bins did not offer that possibility here. Whether the reluctance of the birds to eat the novel food was owing to neophobia or dietary conservatism, the result was protection of the grain.

Unexpectedly (see chapters 2, 3 & 4), there was an effect of odour on the consumption of grain by the birds, even when it was the only novel cue. It is possible that although the birds were using the bins regularly, they were not entirely habituated to them as familiar environments. Rothschild *et al.* (1984) suggested that odour acts as a cue for attention. Once the birds were paying closer attention to their surroundings, they could have become more sensitive to its novelty. If the birds were still wary of the bins, then the addition of a novel odour could have enhanced this wariness. Whatever the cause, it is clear that these birds were sensitive to odours.

As both the NC and NCO treatments were affected by rainwater, it is not safe to draw firm conclusions about the possible interaction of novel colour and odour cues on these finches. It is necessary to conduct further investigations to establish this.

Quality control guidelines in the UK require the prevention of bird contamination from grain stores (Wildey *et al.* 1999). If stored grain were illuminated with coloured (i.e. not white) light, it appears likely that certain avian predators could be dissuaded from frequenting these stores for some time. As non-glass covers are required to protect lights (Wildey *et al.* 1999), these covers could be modified to become coloured filters. This would have the double benefit of reducing grain consumption by the birds, as well as reducing the contamination of the stored crop (by droppings, avian-borne diseases or bird carcasses). The addition of odours to these stores might further deter avian predators, but the grain could potentially become flavoured by them. Grain stored for culinary use, e.g. for bakeries, should not be exposed to such odours. If the grain were being stored for planting, this may not prove disadvantageous. Some workers have found that certain odours are useful additional deterrents against passerines feeding on grain (Avery & Nelms 1990; Nelms & Avery 1997).

'Flat store' grain facilities often have significant amounts of spilt grain around the storage buildings (pers. obs.). Such spillage provides a reasonably large reservoir of familiarly coloured food. Although this serves to attract numerous pest species, the use of coloured lights should make it possible to protect grain in flat stores from avian pests. When familiar-coloured food is available in reasonable abundance, there appears to be little motivation for sampling food of a novel colour even amongst social species. This could prove to be an attractive option for some farmers.

5.6 <u>Conclusions:</u>

The addition of colour to a food by incident light appears to be as effective as coloured dyes at deterring avian predators. This technique could be used to protect stored grain crops. There are also similarities between wild and laboratory-held finches:

- Wild finches are reluctant to eat a familiar food of a novel colour.
- Wild finches can use odour as a cue to assess the familiarity of a food.

Chapter 6

General Discussion

6.1 The Effects of Novel Odour and Novel Colour:

Chicks (chapter 4), zebra finches (chapter 2), some blackbirds (chapter 3) and wild social finches (chapter 5) all possess and use a sense of smell. This supports the view that a bird's olfactory bulb size does not dictate its ability to detect odours (Clark *et al.* 1993). Chicks and zebra finches appear to use their sense of smell to make decisions about the 'safety' of novel food. While blackbirds seem to have this facility (see section 3.9), the majority of individuals do not appear to attend to odour cues. This is an interesting finding, as blackbirds also have the facility to discern relatively small size differences, but do not normally do so (Marples 1993). Perhaps inattention is not detrimental unless there is a clear cost associated with it. From the trials conducted with wild finches, it is not clear whether their detection of odour is used to assess food safety. It is possible that the novel odour was heightening environmental neophobia (Greenberg 1983; Jones 1986).

Comparison of the average reactions of the chicks, zebra finches and blackbirds to novel colour and novel odour cues (Tables 6.1 and 6.2) showed that none of these groups considered familiarly coloured food with a novel odour aversive. There were no significant differences between the C and NO values for either contact or incorporation. There were, however, clear differences between contact (Table 6.1) and incorporation (Table 6.2) values for the C and NC treatments, indicating that a novel colour of a familiar food was aversive. The chicks showed only a small difference in latency to peck at (contact) C and NC foods, but zebra finches took eight times as long to peck at NC food, and blackbirds took about 40 times longer (Table 6.1). The differences were larger when considering consumption (incorporation) of the NC treatment; zebra finches took 10 times longer, and blackbirds about 50 times longer (Table 6.2). Chicks showed a doubling in time of the latency to eat the NC treatment compared with the C treatment. Latency to peck can be considered to be a reflection of the neophobia of the birds, and latency to incorporate a food into the diet as a measure of DC (Marples & Kelly 1999). Consequently, these results indicate that the addition of a novel colour to familiar food increases neophobia and greatly increases DC induced by the food. The combined effect of these two processes causes the birds to avoid the food for long periods. The data from the wild finches (chapter 5), recorded as weight changes, cannot be included in tables 6.1 and 6.2, which lists latencies recorded as the number of presentations. The wild finch data indicates that the novel colour

may be applied by incident light to the familiar food. This information is of potential use to cereal farmers who may need to protect their stored grain from avian pests.

	C	NO	NC	NCO
Chicks	1.0	1.0	1.4 ± 0.2	1.4 ± 0.1
Zebra Finches	1.0	1.0	8.0 ± 3.1	7.7 ± 2.2
Blackbirds	1.0	1.1	43.7 ± 8.8	53.4 ± 9.9

Table 6.1. Mean contact latencies (presentations) for chicks, zebra finches and blackbirds for three novel food types. Novel Odour (NO), Novel Colour (NC) and Novel Colour and Odour (NCO). Control values (C) are given for comparison. Standard errors are given if they are greater than or equal to 0.1.

	C	NO	NC	NCO
Chicks	1.2	1.2	2.7 ± 1.1	6.8 ± 1.4
Zebra Finches	1.3 ± 0.3	2.0 ± 0.5	13.0 ± 2.0	29.4 ± 4.1
Blackbirds	1.2 ± 0.1	1.3 ± 0.1	61.3 ± 10.1	61.9 ± 9.8

Table 6.2. Mean incorporation latencies (presentations) for chicks, zebra finches and blackbirds for three novel food types. Novel Odour (NO), Novel Colour (NC) and Novel Colour and Odour (NCO). Control values (C) are given for comparison. Standard errors are given if they are greater than or equal to 0.1.

While the chicks, and zebra finches, showed a synergistic effect in their latencies to incorporate food with both a novel colour and a novel odour (NCO), the blackbirds did not. This appears to be owing to the blackbirds' inattention to odour cues. A few individual blackbirds did appear to attend to the odour cues of the NCO treatment, and as a result treated it more cautiously than the NC food (chapter 3). An important observation regarding the behaviour of both chicks and some blackbirds towards odour was that odour did not need to be novel; even a familiar odour appeared to generate a synergistic latency to contact a familiar food of novel colour. Previous research (Rowe & Guilford 1999a) has not observed this behaviour with familiar odours. The birds may have been assessing the odours by relative concentration, and so were only attending to them when they were at higher concentrations (i.e. at the site of the NCO treatment, and thus in the presence of novel coloured food).

An analysis of the data in table 6.1 reveals several clear trends. Using latency as the dependent variable, and novel colour, species and DC (incorporation of the food) as fixed factors, novel colour (univariate ANOVA: $F_{1, 12} = 200.6$, $p < 0.001$), species ($F_{2, 12} = 94.0$, $p < 0.001$) and DC ($F_{1, 12} = 10.0$, $p < 0.01$) all had main effects. There are also interactions between novel colour and DC ($F_{1, 12} = 8.7$, $p < 0.05$), and novel colour and species ($F_{2, 12} = 94.2$, $p < 0.001$). The main effect of novel colour means that a familiar food with a novel colour was either contacted or incorporated differently than a familiar food of familiar colour. The interaction effect of novel colour and species indicates that different species behaved differently to familiar food of novel colour.

The main effect of species cannot be explained clearly. Differences between species may reflect differences in test environments (in the field or the laboratory), rearing conditions (in the field or the laboratory), age (juvenile or adult), avian order (*Galliformes* or *Passeriformes*) and species. The neophobic aspect of the chicks' behaviour may be disproportionately small owing to their peculiar feeding and exploratory techniques (Martaresche *et al.* 2000). This could explain the main effect of species at a higher taxonomic level. It appears that both the age of the subjects and the environment in which they were tested may have had a significant effect on their behaviour. Previous work has found that young birds show little or no neophobia or DC towards a prey that their parents clearly avoid (Beissinger *et al.* 1994). Beissinger and his colleagues (1994) found that juvenile snail kites did not display a preference for the snails that they fed on, whereas their parents showed a strong preference for familiar *Pomacea doliodes* snails over an unfamiliar species (*Pomacea urceus*). Both blackbirds and robins showed similar behaviour (section 3.7). Another possible interpretation of the species differences detailed above is that some of these species were wild, whereas others were captive. Wild-caught individuals have been demonstrated to show considerably greater neophobic responses than laboratory held individuals of the same species (Mitchell 1976). Either way, it appears that experience, by age or environment, may have a large effect on the feeding behaviour of animals. It is not possible to draw firm conclusions, given such a small group of ages and species; Of the birds I tested, however, young birds in the laboratory were less neophobic than older birds in the laboratory, which in turn were less neophobic than older birds in the wild. The same trend was true for DC. By extending these studies to encompass a wider range of bird and other animal species, it would be possible to test the generality of these findings.

The main effect of DC indicates that across the species considered, there was a significant difference between the time taken to contact a treatment and incorporate it into the diet. These differences were only slight for the C and NO treatments, but more pronounced for the NC and NCO treatments. My work (chapter 3), and previous work (Marples *et al.* 1998) have shown that both neophobia and DC are highly variable within wild populations. These studies have also shown however, that the time taken to incorporate a familiar food of novel colour is significantly longer than the time taken to contact it. This supports the claim that neophobia and DC are discrete processes (Marples & Kelly 1999).

6.2 <u>The Process of Incorporation of a Novel Food into the Diet:</u>

The process of incorporation of a new food into the diet is composed of several stages (Figure 6.1). These stages appear to provide several opportunities for a bird to show preference over a novel food in favour of a familiar one. Some of the stages within the incorporation process have not been fully appreciated previously. The initial reluctance to approach a novel food (figure 6.1 step 1) has been described as neophobia (section 1.4.2). The subsequent initial contact and sampling (figure 6.1 steps 2 and 3) of that food prior to regular eating could be considered analogous to 'learned safety' (Kalat & Rozin 1973). Learned safety is the process whereby an animal learns to associate no ill effects with the consumption of a new food. When a bird is regularly eating a food, it may differentially or non-differentially incorporate it (section 3.7). This means that a bird can still discriminate against a prey type, despite having added it to its diet. So, from first encountering a novel food to non-differentially incorporating that food, the bird must progress through at least four stages of acceptance (figure 6.1). All of these stages present the opportunity to reject the prey.

It appears that the two key aspects of dietary incorporation are neophobia and DC. Although a number of authors have combined these two behaviours, my work shows there is strong evidence to suggest that they are quite distinct. Figure 6.1 represents neophobia as step 1, while steps 2 to 4 represent DC. Birds that show high levels of DC towards a food type may not show high levels of neophobia towards it. Chicks showed relatively low levels of neophobia towards NCO food, but relatively high levels of DC (Table 6.1 and
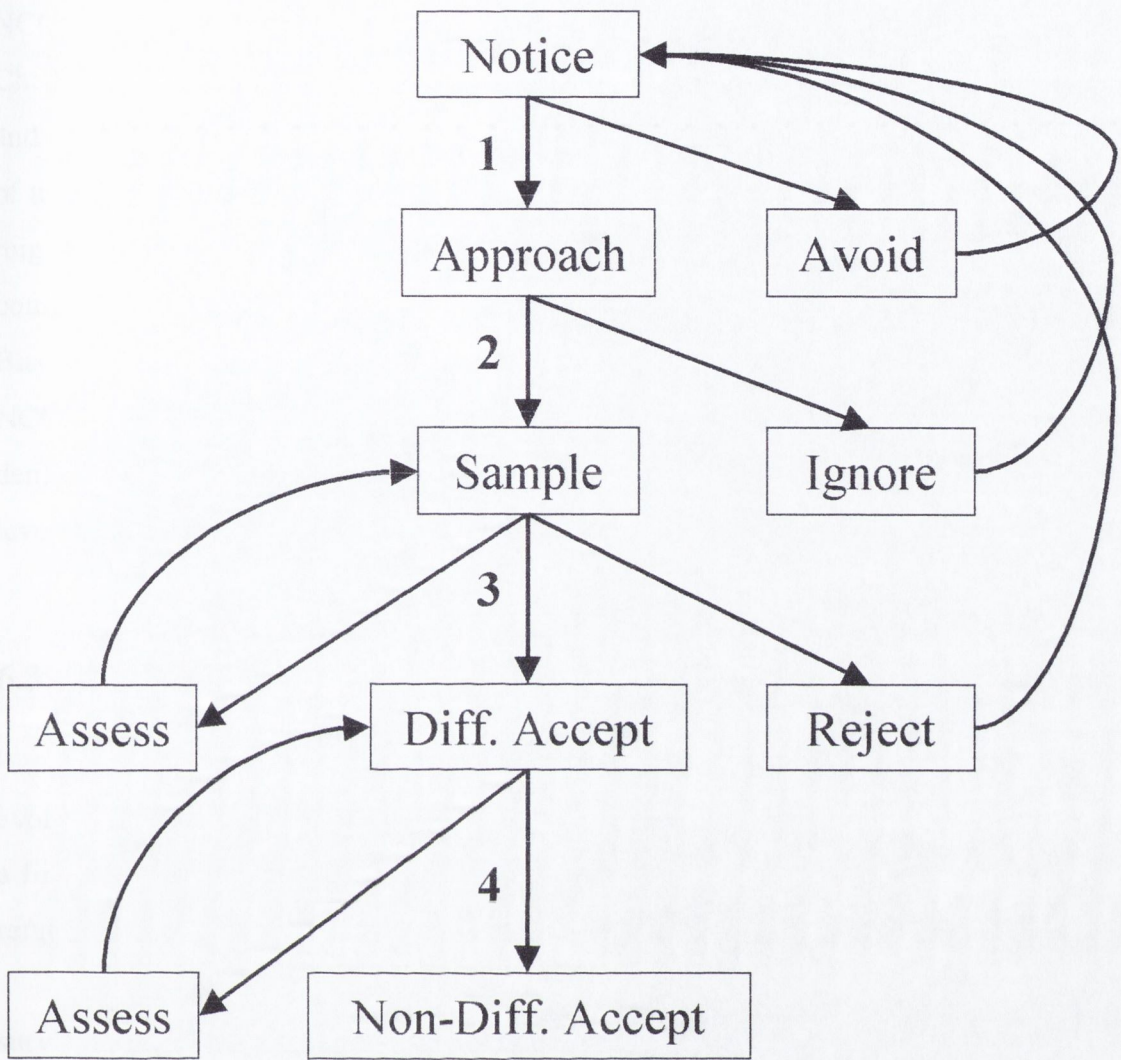
Figure 6.1. A schematic representation of the four steps (numbered) leading from initial encounter to non-differential incorporation of a novel food by a bird.

Chapter 4). Zebra finches showed similarly increased neophobia towards both NC and NCO foods, but relatively greater DC towards NCO food than NC food (Table 6.1 and Chapter 2). Blackbirds showed similar levels of both neophobia and DC towards both NC and NCO foods (Tables 6.1 & 6.2 and Chapter 3). As DC appears to comprise a larger part of the acceptance process (Figure 6.1 steps 2 to 4) than neophobia (Figure 6.1 step 1), one might predict that the DC component of latencies would be larger than the neophobic components. This trend appears to depend on the attention given to the novel cues. Blackbirds, being generally inattentive to novel odour cues, did not show this trend for NCO food, whereas chicks and zebra finches did (Tables 6.1 & 6.2). The wild finches demonstrated sensitivity for odours (Chapter 5), so might also be expected to show high levels of DC.

6.3 <u>The Importance of DC in the Evolution of Aposematism:</u>

Guilford (1990) discusses the possible pathways by which an aposeme could evolve. None of these potential pathways allows for the initial evolution of novel colour as a first step. The following proposed evolutionary pathway of an aposeme is not meant to refute any of the previously discussed routes (Guilford 1990), but simply to augment them.

I believe that the neophobic and DC behaviours of adult birds could allow for the survival of novel coloured morphs of a familiar prey. Perhaps it is possible that such behaviours might radically affect local populations. Recent work on avian predators (R. Thomas *et al.* unpublished data) has demonstrated that it is possible to 'evolve' a novel coloured and palatable population from a familiar coloured and palatable one, over a relatively short time scale. A novel coloured morph (a red or green pastry bait) was introduced into a familiar population (brown baits – made from equal parts red and green dye) of 20 palatable prey items. Such populations were presented to 10 individually housed, wild caught robins. At the end of the feeding day, the ratio of the two morphs was calculated. Using these proportions, the population for the next day was generated. If the birds failed to eat the new morphs, they rapidly became more numerous. The individual trials concluded when either the new morph had become extinct or reached fixation (population now composed solely of new morph). A second version of this experiment was conducted using novel blue and yellow morphs 'evolving' from a familiar green one (made from equal parts blue and yellow dye). The researchers found that classical warning colours

(red and yellow) were more likely to evolve to fixation (6/10 trials), but that it was occasionally possible (1/10 trials) with traditionally neutral colours (blue and green). It appears that if a novel colour mutation arose in a familiar prey species, it might afford considerable protection for the new morphs. These results cannot hope to be conclusive as they were conducted on captive birds, and used a highly accelerated 'generation' period. None the less the information adds further weight to the importance of DC.

As these novel colour morphs have such robust protection, one might predict other conspicuous prey to be similarly protected. Pfennig *et al.* (2001) demonstrated that Batesian mimics (brightly coloured and palatable prey) could only survive if their models were also present. They used tricolour ringed pattern plasticine replicas of the local kingsnakes (mimics of the poisonous coral snakes) along with striped (control) morphs and plain brown (control) morphs. They arranged a group of three replicas (one of each morph) in an area of two-metre radius. At each test site, they placed 10 of these 'triplets' along a transect line at 75m intervals. Tests were conducted at eight sympatric (coral snake species present) sites and eight allopatric (coral snake species absent) sites. They found that the kingsnake replicas were attacked on significantly more occasions in the areas of allopatry.

The protection of the mimics was shown to be reliant on the aversion learning associated with the Batesian models. As the novel coloured prey I used were not unprofitable to their predators (or mimics of unprofitable prey), aversion learning was not involved in their rejection. The rejection of the prey was primarily due to their novelty. The novel coloured prey I used were a new morph of a familiar prey. Psychological studies have suggested that objects combining some familiar and some novel cues are actually more 'frightening' than a totally novel object (Hebb 1946). This finding is supported by the effect of experience on the reactions of blackbirds (section 6.1) to novel coloured prey, and snail kites (Beissinger *et al.* 1994) to prey of a novel weight and texture.

If a novel colour morph can be established, it seems reasonable to assume it may be maintained, at least at a low frequency. The reactions of blackbirds to a familiar food of novel colour indicate that DC may persist for several months (Marples *et al.* 1998). My own studies (chapter 3) have shown that some birds may refuse to sample novel coloured morphs for over a year. Two passerine species (blackbirds and zebra finches) demonstrated a robust memory for food colour over periods of time that would allow seasonal re-emergence of insect species. These memories appeared to be equally robust for both previously incorporated and unincorporated foods. This indicates that DC may be

maintained for considerable periods of time. It is conceivable that at least some of the experienced predators would never sample the novel coloured morphs, even if they encountered them frequently. Despite the protection afforded by the DC of experienced predators, some 'unfamiliarised' predators may still sample the novel morphs. Over time the number of experienced predators will diminish as they die and are replaced by 'unfamiliarised' juveniles. If the novel morph is clumped in its distribution, however, it may effectively generate experienced predators in each succeeding generation. The novel morph will occur in a relatively localised area, falling in a relatively low number of feeding areas. So the majority of predators will be familiar with the high frequency morph only. Under such conditions, the number of experienced predators would be maintained at a high level, and the number of 'unfamiliarised' predators at a low level. If the low-frequency novel morph occurs alongside the high-frequency familiar morph, and prey density is not extremely high, then apostatic selection (Section 1.4) will dictate that the familiar morph is predated, and the novel morph disregarded. Apostatic selection effectively generates experienced predators, and protects the novel morph. This reduces concerns about the generation times of predators relative to their prey, as each new generation of predators will naturally favour apostatic selection. Such 'evolutionary assistance' for the aposeme by apostatic selection could occur in both clumped and unclumped populations.

Allowing for the evolution of an unprotected conspicuous morph, consider a larval population that has two morphs, one a cryptic morph at a high frequency and one a conspicuous morph at a low frequency. If this larva incorporates a new food plant, for example, it may gain some protective benefit from the compounds within that plant (Camara 1997; Hartmann *et al.* 1999). Now we have a low frequency defended and advertised (i.e. aposematic) morph, and a high frequency defended and cryptic morph. The aposematic morph may still be protected from experienced predators, and will now gain protection from 'unfamiliarised' predators through aversion learning. All predators would learn to avoid the aposematic morph rapidly (Roper & Wistow 1986; Roper & Redston 1987), but may be somewhat slower to learn about the cryptic morphs (Roper & Wistow 1986; Roper & Redston 1987). These would be favourable conditions for the aposematic morph to proliferate in the population. The aposematic morph would become established as the common form, and we would have our aposeme. Aggregation may further refine the protection and enhance the survival of the aposeme.

The survival of the initially unprotected novel morph is key to the first step of the evolutionary scenarios mentioned above. This in turn relies on these prey surviving encounters with 'unfamiliarised' predators. It has yet to be satisfactorily demonstrated that this could happen over an evolutionary time scale. If the numbers of 'unfamiliarised' predators were sufficiently low, or their 'training' through apostatic selection was sufficiently rapid, the novel prey should survive in small numbers.

6.4 The Importance of DC in the Evolution of Aposematic Polymorphism:

Another situation that appears difficult to explain is why certain species that have successfully developed aposematism should change their colour pattern. The occurrence of polymorphic aposemes is well known (Mallet & Joron 1999). The last section speculated about an aposeme that had the potential to aggregate. Once an aggregated aposeme exists, further developments are possible. Both neophobia and DC would immediately protect any novel coloured morphs of the aposeme. Greenwood *et al.* (1989) found that a rare morph within an aposematic population is at less of a frequency-dependent disadvantage when its distribution is clumped rather than randomly mixed with the common form. The rare morph effectively increases its local density by clumping, and so has a local density equivalent to the common morph in other areas. So clumping of a new rare aposematic morph could effectively switch the local balance, and turn it into the common form within the foraging patch. Such a system allows the replacement of one aposematic form with another within an area, providing the new morph is at no disadvantage over the established one (Greenwood *et al.* 1989). By adding protection to the new morph, neophobia and DC would effectively promote polymorphism within the species.

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Appendix 1

Bird	Control contact	Control incorp	Pyrazine contact	Pyrazine incorp	Red contact	Red incorp	Red/Pyr contact	Red/Pyr incorp
F BBR	1	1					5	22
F BNR	1	1					3	36
F YNR	1	1					19	20
M BRBL							2	26*
M YBR							6	26*
F BR			1	1	6	6		
F MBL			1	1	11	14		
F RL			x	x	16	17		
F RR			1	1	3	9		
F YL			x	x	5	9		
M BWR			1	1	12	29		
M BYR			1	1	11	25		
M MBL			x	x	3			
M NBL			x	x	1	12		
M YYR			1	1	4	6		
F MMR	1	1					8	60
F NWL	1	1					x	x
F OR	1	1					x	x
M MBR	1	1					1	27*
M MWR	1	1					7	26*
M WYR	1	4					18	22*
F BL			1	2	5	9		
F ML			1	1	3	5		
F MYR			1	1	7	12		
F NR			1	1	x	x		
F NWL			1	1	x	x		
F WL			1	1	x	x		
M GLR			1	7	8	13*		
M ML			1	4	x	x		
M MMR			1	4	1	13		
M MYR			1	1	x	x		
M RR			1	1	x	x		
M WR			1	2	x	x		
M YL			1	3	x	x		
M YMR			1	3	1	13*		

Latencies (presentations) for individual zebra finches. Values marked with an asterisk are Minimum Possible Values.

Bird	Control		Pyrazine		Red		Red/Pyr	
	contact	incorp	contact	incorp	contact	incorp	contact	incorp
F BBR	4	4					4,120	22,951
F BNR	1	1					1,830	60,350
F YNR	5	5					24,300	24,310
M BRBL							1,360	46,800*
M YBR							8,110	46,800*
F BR			1	1	7,480	7,480		
F MBL			3	3	12,920	15,508		
F RL			x	x	14,800	14,893		
F RR			1	1	1,951	6,652		
F YL			x	x	1,502	3,845		
M BWR			1	1	14,350	30,891		
M BYR			2	2	13,452	32,341		
M NBL			x	x	48	3,904		
M YYR			4	4	3,820	3,841		
F MMR	6	6					8,160	75,330
F NWL	2	2					x	x
F OR	1	1					x	x
M MBR	4	4					100	46,920*
M MWR	1	1					9,735	28,937*
M WYR	5	5					39,600	39,600*
F BL			2	2	3,140	8,907		
F ML			6	6	3,303	3,342		
F MYR			7	7	14,820	15,852		
F NR			3	3	x	x		
F NWL			5	5	x	x		
F WL			2	2	x	x		
M GLR			7	7	13,020	19,310*		
M MBL			x	x	2,907	7,800*		
M ML			10	10	x	x		
M MMR			8	8	870	14,428		
M MYR			4	4	x	x		
M RR			3	3	x	x		
M WR			3	3	x	x		
M YL			4	4	x	x		
M YMR			7	7	400	19,840*		

Latencies (s) for individual zebra finches. Values marked with an asterisk are Minimum Possible Values.

Appendix 2

Statistical Modelling of Blackbird Data

In order to calculate the confidence intervals for the different presentation regimes, a theoretical collection of bait selection orders was constructed (detailed below). This collection allowed for all possibilities where the two baits from any single treatment (F = familiar, N = 1st novel treatment, O = 2nd novel treatment) were eaten consecutively (as this accounted for 96% of all presentations). Taking this data set as our random distribution, the standard errors of the mean were calculated for 5- and 10-point moving averages (i.e. sample sizes of 5 and 10). Using these standard errors, the 95% and 99% confidence intervals (CIs) were calculated. These CIs were then used to decide whether the choices of a blackbird were different from a purely random choice of baits. When considering a random variation from the 95% CI, the blackbird treatment preference line was only allowed to cross it once every 20 points (i.e. 5% of the time). When considering a random variation from the 99% CI, the blackbird treatment preference line was only allowed to cross it once every 99 points (i.e. 1% of the time).

6 baits

N	N	F	F	F	F	Av Nov	Av Fam	Diff
1	2	3	4	5	6	1.5	4.5	-3
2	3	1	4	5	6	2.5	4	-1.5
3	4	1	2	5	6	3.5	3.5	0
4	5	1	2	3	6	4.5	3	1.5
5	6	1	2	3	4	5.5	2.5	3

Average	0	
StDev	2.12132	
SEM - 5	0.948683	
SEM -10	0.67082	

		5pt	10pt
CI 95%	1.959961	1.8594	1.3148
CI 99%	2.575835	2.4437	1.7279

8 baits

N	N	O	O	F	F	F	F	Av Nov	Av Fam	Diff
1	2	3	4	5	6	7	8	1.5	6.5	-5
1	2	4	5	3	6	7	8	1.5	6	-4.5
1	2	5	6	3	4	7	8	1.5	5.5	-4
1	2	6	7	3	4	5	8	1.5	5	-3.5
1	2	7	8	3	4	5	6	1.5	4.5	-3
2	3	4	5	1	6	7	8	2.5	5.5	-3
2	3	5	6	1	4	7	8	2.5	5	-2.5
2	3	6	7	1	4	5	8	2.5	4.5	-2
2	3	7	8	1	4	5	6	2.5	4	-1.5
3	4	1	2	5	6	7	8	3.5	6.5	-3
3	4	5	6	1	2	7	8	3.5	4.5	-1
3	4	6	7	1	2	5	8	3.5	4	-0.5
3	4	7	8	1	2	5	6	3.5	3.5	0
4	5	1	2	3	6	7	8	4.5	6	-1.5
4	5	2	3	1	6	7	8	4.5	5.5	-1
4	5	6	7	1	2	3	8	4.5	3.5	1
4	5	7	8	1	2	3	6	4.5	3	1.5
5	6	1	2	3	4	7	8	5.5	5.5	0
5	6	2	3	1	4	7	8	5.5	5	0.5
5	6	3	4	1	2	7	8	5.5	4.5	1
5	6	7	8	1	2	3	4	5.5	2.5	3
6	7	1	2	3	4	5	8	6.5	5	1.5
6	7	2	3	1	4	5	8	6.5	4.5	2
6	7	3	4	1	2	5	8	6.5	4	2.5
6	7	4	5	1	2	3	8	6.5	3.5	3
7	8	1	2	3	4	5	6	7.5	4.5	3
7	8	2	3	1	4	5	6	7.5	4	3.5
7	8	3	4	1	2	5	6	7.5	3.5	4
7	8	4	5	1	2	3	6	7.5	3	4.5
7	8	5	6	1	2	3	4	7.5	2.5	5

Average	0	
StDev	2.798809	
SEM - 5	1.251666	
SEM -10	0.885061	

		5pt	10pt
CI 95%	1.959961	2.4532	1.7347
CI 99%	2.575835	3.2241	2.2798

Appendix 3

Bird	Total	CC	CI	NOC	NOI	NCC	NCI	NCOC	NCOI
FBGBL	39	1	1	1	1	7	7	7	7
FBMMR	37	1	1	1	1	38*	38*	38*	38*
FBRRL	34	1	1	2	2	35*	35*	35*	35*
FBRWR	75	1	1	1	1	7	76*	76*	76*
FB-SB	5	1	1	1	1	6*	6*	6*	6*
FBWMR	4	1	1	1	1	5*	5*	5*	5*
FBWR	26	1	1	1	1	2	27*	27*	27*
MBBYL	31	1	1	1	1	32*	32*	32*	32*
MBGL	54	1	1	1	1	31	55*	31	55*
MBGRL	2	1	3*	1	3*	3*	3*	3*	3*
MBRRL	33	1	1	1	1	8	8	8	8
MB-SC	23	1	1	1	1	24*	24*	24*	24*
MBWBL	9	1	1	1	4	10*	10*	10*	10*
MBWRL	64	1	1	1	1	65*	65*	65*	65*
MBWYL	40	1	4	1	1	41*	41*	14	41*
MBWYR	87	1	1	1	1	88*	88*	88*	88*
MBYML	65	1	1	1	1	66*	66*	13	66*

Latencies (presentations) for individual blackbirds to contact and incorporate the different treatments during the 1998 field season (protocol 1 chapter 3). Values marked with an asterisk are Minimum Possible Values.

Bird	Total	CC	CI	NOC	NOI	NCC	NCI	NCOC	NCOI
FB4u1	9	1	1	1	1	1	1	1	1
FB4u2	56	1	1	1	1	1	57*	1	57*
FB4u3	96	1	1	1	1	1	97*	55	97*
FB4uE	5	1	1	1	1	6*	6*	6*	6*
FB4u5	104	1	1	1	1	45	60	9	48
FB4u6	165	1	1	1	3	16	30	73	166*
FB4u8	21	1	1	1	1	22*	22*	22*	22*
FB4uS	37	1	4	1	1	9	16	9	11
FBMBR	19	1	1	2	2	20*	20*	20*	20*
FBMWL	35	1	1	2	5	36*	36*	36*	36*
FBRBL	5	1	3	1	6*	6*	6*	6*	6*
FBRRL	213	1	1	1	1	26	214*	95	95
FBRWR	147	1	1	1	1	39	39	35	39
FBWMR	112	1	1	1	1	104	113*	113*	113*
FBWR	98	1	1	1	1	99*	99*	99*	99*
MB5u6	205	1	1	1	1	206*	206*	206*	206*
MB6u3	28	1	1	2	2	29*	29*	29*	29*
MB6u6	118	1	1	1	1	4	4	32	32
MB6uN	5	2	2	1	1	6*	6*	6*	6*
MBBYL	222	2	2	1	1	223*	223*	223*	223*
MBGL	283	1	1	1	1	284*	284*	284*	284*
MBGRL	104	1	1	1	1	78	78	78	78
MBMR	23	1	1	1	1	24*	24	24	24
MBMWR	40	1	1	1	1	27	27	25	25
MBNL	34	1	1	1	1	35*	35*	11	35*
MBRRL	75	1	1	1	1	7	57	7	57
MBRYL	1	2*	2*	1	2*	2*	2*	2*	2*
MBWBL	191	1	1	1	4	192*	192*	192*	192*
MBWRL	133	1	1	1	1	53	134*	134*	134*
MBWYL	52	1	1	1	1	53*	53*	53*	53*
MBYML	245	1	1	1	1	18	246*	246*	246*
B3Ju	3	1	1	2	4*	2	4*	2	4*

Latencies (presentations) for individual blackbirds to contact and incorporate the different treatments during the 1999 and 2000 field seasons (protocol 2 chapter 3). Values marked with an asterisk are Minimum Possible Values.

Bird	yr	stage 1	stage 2	stage 3	stage 4
FBGBL	98	7	7	7	13
FBMMR	98	38*			
FBRRL	98	35*			
FBRWR	98	7	76*		
FB-SB	98	6*			
FBWMR	98	5*			
FBWR	98	2	27*		
MBBYL	98	32*			
MBGL	98	31	31	55*	
MBGRL	98	3*			
MBRRL	98	8	8	8	11
MB-SC	98	24*			
MBWBL	98	10*			
MBWRL	98	65*			
MBWYL	98	41*			
MBWYR	98	88*			
MBYML	98	66*			
FB4u1	99	1	1	1	1
FB4u2	99	1	1	57*	
FB4u3	99	1	97*		
FB4uE	99	6*			
FB4u5	99	45	45	60	85
FB4u6	99	16	16	30	42
FB4u8	99	22*			
FB4uS	99	9	9	16	16
FBMBR	99	20*			
FBMWL	99	36*			
FBRBL	99	6*			
FBRRL	99	26	214*		
FBRWR	99	39	39	39	53
FBWMR	99	104	113*		
FBWR	99	99*			
MB5u6	99	206*			
MB6u3	99	29*			
MB6u6	99	4	4	4	7
MB6uN	99	6*			
MBBYL	99	223*			
MBGL	99	284*			
MBGRL	99	78	78	78	79
MBMR	99	24*			
MBMWR	99	27	27	27	30
MBNL	99	35*			
MBRRL	99	7	7	57	59
MBRYL	99	2*			
MBWBL	99	192*			
MBWRL	99	53	134*		
MBWYL	99	53*			
MBYML	99	18	246*		
B3Ju	99	2	2	4*	

The various stages of dietary incorporation for the NIC treatment by individual blackbirds. Values marked with an asterisk are Minimum Possible Values.

Bird	yr	stage 1	stage 2	stage 3	stage 4
FBGBL	98	7	7	7	40*
FBMMR	98	38*			
FBRRL	98	35*			
FBRWR	98	76*			
FB-SB	98	6*			
FBWMR	98	5*			
FBWR	98	27*			
MBBYL	98	32*			
MBGL	98	31	55*		
MBGRL	98	3*			
MBRRL	98	8	8	8	9
MB-SC	98	24*			
MBWBL	98	10*			
MBWRL	98	65*			
MBWYL	98	14	41*		
MBWYR	98	88*			
MBYML	98	13	66*		
FB4u1	99	1	1	1	2
FB4u2	99	1	1	57*	
FB4u3	99	55	55	97*	
FB4uE	99	6*			
FB4u5	99	9	48	48	49
FB4u6	99	73	73	166*	
FB4u8	99	22*			
FB4uS	99	9	9	11	13
FBMBR	99	20*			
FBMWL	99	36*			
FBRBL	99	6*			
FBRRL	99	95	95	95	214*
FBRWR	99	35	35	39	39*
FBWMR	99	113*			
FBWR	99	99*			
MB5u6	99	206*			
MB6u3	99	29*			
MB6u6	99	32	32	32	41
MB6uN	99	6*			
MBBYL	99	223*			
MBGL	99	284*			
MBGRL	99	78	78	78	78
MBMR	99	24*			
MBMWR	99	25	25	25	25
MBNL	99	11	35*		
MBRRL	99	7	7	57	57
MBRYL	99	2*			
MBWBL	99	192*			
MBWRL	99	134*			
MBWYL	99	53*			
MBYML	99	246*			
B3Ju	99	2	2	4*	

The various stages of dietary incorporation for the NCO treatment by individual blackbirds.
Values marked with an asterisk are Minimum Possible Values.

Appendix 4

	Group 1					Group 2			
chick	C	NO	NC	NCO	chick	C	NO	NC	NCO
B1	1	1	1	1	B3	1	1	3	1
B1B2	1	1	1	1	B3B2	1	1	1	1
B1B5	1	1	1	1	B3B4	1	1	1	1
B1G2	1	1	1	1	B3G2	1	1	1	1
B1N2	1	1	1	1	B3G4	1	1	1	1
B1R2	1	1	1	1	B3N2	1	1	2	1
B2	1	1	1	1	B3R4	1	1	1	1
B5	1	1	1	1	B4	3	1	3	1
G1	1	1	1	1	G3	1	1	1	1
G1B2	1	1	1	1	G3B2	1	1	1	1
G1G2	1	1	1	2	G3B4	2	1	2	3
G1G5	1	1	1	1	G3G4	1	1	1	1
G1N2	1	1	1	1	G3G5	1	1	1	11
G1N5	1	1	4	1	G3N2	1	1	1	2
G1R2	1	1	1	1	G3N4	1	1	3	1
G1R5	1	1	6	1	G3R2	1	1	1	1
G2	1	1	1	1	G3R4	1	1	1	2
N1	1	1	1	1	N3	1	1	1	1
N1B2	1	1	1	1	N3B4	1	1	1	1
N1N2	1	1	1	1	N3N4	1	1	1	1
N1R2	1	1	1	1	N3R4	3	3	3	3
N2	1	1	1	2	N4	1	1	1	2
R1B2	1	1	1	1	R3B4	1	1	1	1
R1N2	1	1	1	1	R3N4	1	1	1	1
R1R2	1	1	1	1	R3R4	1	1	1	5
R2	1	1	4	1	R4	1	1	1	1

Latency (presentations) of individual chicks within the two experimental groups to contact the four food treatments. Values marked with an asterisk are Minimum Possible Values.

	Group 1					Group 2			
chick	C	NO	NC	NCO	chick	C	NO	NC	NCO
B1	1	1	15*	15*	B3	1	1	11*	11*
B1B2	1	1	1	1	B3B2	1	1	1	1
B1B5	1	1	13*	13*	B3B4	1	1	11*	11*
B1G2	1	1	15*	6	B3G2	1	1	1	11*
B1N2	1	1	1	1	B3G4	1	1	1	11*
B1R2	1	1	3	15*	B3N2	1	1	3	11*
B2	1	1	1	15*	B3R4	1	1	3	1
B5	1	1	15*	11	B4	3	3	3	3
G1	1	1	6	15*	G3	1	1	1	5
G1B2	1	1	3	7	G3B2	1	1	1	3
G1G2	1	1	15*	15*	G3B4	2	1	2	11*
G1G5	-	-	-	-	G3G4	1	1	1	11*
G1N2	1	1	1	1	G3G5	1	1	11*	11*
G1N5	1	1	5	7	G3N2	1	1	11*	11*
G1R2	1	1	1	1	G3N4	4	1	11*	11*
G1R5	1	1	15*	15*	G3R2	1	1	11*	11*
G2	1	1	15*	15*	G3R4	1	4	11*	11*
N1	1	1	15*	15*	N3	1	1	1	11*
N1B2	1	1	1	1	N3B4	1	1	11*	11*
N1N2	1	1	1	1	N3N4	1	1	11*	11*
N1R2	1	1	1	15*	N3R4	3	3	11*	11*
N2	1	2	5	6	N4	1	1	1	8
R1B2	1	1	1	1	R3B4	1	1	1	11*
R1N2	1	1	1	1	R3N4	-	-	-	-
R1R2	1	1	15*	15*	R3R4	1	1	3	7
R2	1	1	15*	15*	R4	4	4	11*	11*

Latency (presentations) of individual chicks within the two experimental groups to incorporate the four food treatments. Values marked with an asterisk are Minimum Possible Values.

Appendix 5

Date	Site 1				Site 2			
	C	NO	NC	NCO	C	NO	NC	NCO
baseline	1000	1000	1000	1000	1000	1000	1000	1000
13.12.99	985	990	995	1000	1000	1000	1010	1015
14.12.99	985	980	995	1010	990	995	1005	1010
15.12.99	975	985	995	1010	990	995	1005	1020
16.12.99	970	980	995	1010	990	995	1005	1020
17.12.99	960	975	990	1015	985	995	1005	1020
18.12.99	960	965	990	1010	980	1000	1000	1015
19.12.99	965	965	990	1015	980	995	1000	1015
20.12.99	955	970	995	1010	965	1005	995	1020
21.12.99	955	970	995	1010	965	1005	995	1010
22.12.99	945	960	1005	1010	960	1000	1000	1020

Actual grain weights (g) of the four treatment groups at the two sites in the Wexford Slobs, during the 10-day feeding trial.