UNIVERISTY OF SOUTHAMPTON

# FREQUENCY-DEPENDENT SELECTION AT HIGH PREY DENSITY, WITH EMPHASIS ON THE EFFECT OF PALATABILITY

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## ABSTRACT

FACULTY OF SCIENCE

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# FREQUENCY-DEPENDENT SELECTION AT HIGH PREY DENSITY, WITH EMPHASIS ON THE EFFECT OF PALATABILITY

by Khaled Al-Rasbi

Anti-apostatic selection (selection against the rare form) has been demonstrated in several studies that used high density of dimorphic artificial prey. Although anti-apostatic selection has been proposed to be one of the factors that has led to distasteful prey usually being monomorphic, its effect on distasteful prey when at a high density has not been demonstrated. Moreover, the interaction between frequency and palatability, if any, is not clear. The experiments in much of the thesis attempted to determine (1) whether at high density of distasteful artificial prey, wild birds and exotic captive birds do exert anti-apostatic selection and (2) whether there is an effect of palatability on the strength and direction of frequency-dependent selection. In another part of the thesis, an attempt was made to investigate whether there was a detectable effect of anti-apostatic selection on the dense intertidal polymorphic snail *Umbonium vestiarium.*

Experiments with wild birds and captive exotic birds feeding on high density of dimorphic pastry baits revealed that there is a tendency for selection on distasteful prey to be anti-apostatic whether the colours of the baits were red and yellow (common colours for aposematic prey) or green and brown (common colour for cryptic edible prey), and whether the design was fixed frequency (one level of frequency per site) or fixed palatability (one level of palatability per site). There was no significant effect of palatability.

In a separate set of experiments, data were obtained for known species of free ranging wild birds and individuals. There was evidence for frequency-dependent selection but the effect of palatability was inconsistent.

Two experiments were carried out to test whether wild birds would feed on anti-apostatic manner on blowfly maggots *Calliphora vomitoria* and whether selection is affected by palatability. The results demonstrated that selection was anti-apostatic in both experiments (fixed frequency and fixed palatability). Selection was affected by palatability.

A field experiment on the intertidal polymorphic snail *Umbonium vestiarium* in four beaches in Oman showed that although both the density and diversity of the snail varied among the four beaches, there was no significant interaction between density and diversity. This is contrary to the prediction from the hypothesis that selection should be anti-apostatic on polymorphic populations at such high densities. An experiment with square pastry baits suggested that heterosematism (when the colour of the underside is different from the colour of the upperside) promotes apostatic selection (selection against common form).

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# Chapter 1

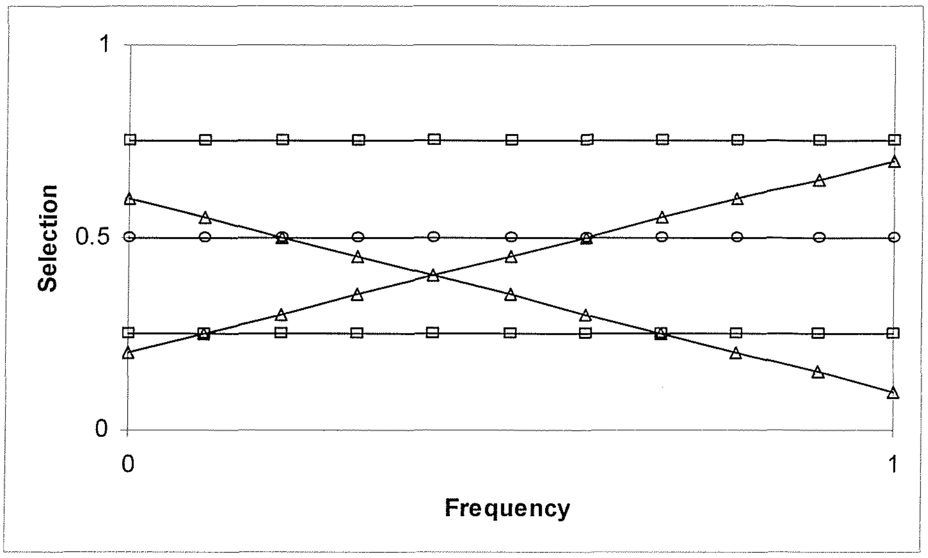
# Introduction

## *1-1- Frequency-dependent selection by predators*

Selection by predators can be either independent of frequency of the different types of prey, or dependent of frequency (Clarke, 1962) (figure 1). Disproportionate predation on commoner types of food can maintain the diversity of the prey population and therefore has attracted biologists who refer to it as 'switching' (Murdoch, 1969) or 'apostatic selection' (Clarke, 1962). Many different kinds of processes, not just predation, can create frequency-dependent selection and, as Heino *et al.* (1998) have mentioned, there are a similar number of definitions.

Frequency-dependent selection occurs in a wide range of hunting animals (Clarke, 1962, 1975; Allen, 1972, 1974, 1976; Harvey *et al.*, 1974; Ayala & Campbell, 1974; Murdoch & Oaten, 1975; Greenwood & Elton, 1979; Horsley *et al.*, 1979; Barret, 1988). Under some conditions, fish (Popham, 1942 reanalysed by Clarke, 1962; Hobson, 1968; Maskell *et al.*, 1977; Ohguchi, 1978, 1981), predatory birds, mammals (Greenwood *et al.*, 1984a, 1984b; Church *et al.*, 1996) and arthropods (Sherratt & Harvey, 1993) seem to concentrate on the common types of prey and overlook rare types. Many of these predators hunt by sight and should therefore be capable of maintaining colour polymorphism (see section 1.2) in their prey populations (Clarke, 1962; Moment, 1962; Owen, 1963, 1965, 1969; Greenwood, 1969; Croze, 1970; Allen, 1974; Bond & Kamil, 2002). In addition, mammals hunting by smell should maintain olfactory polymorphism (Greenwood *et al.*, 1984a, 1984b; Church *et al.*, 1996).

**Figure 1-1:** Selection can be independent of prey frequency (horizontal lines □) or dependent on frequency (negative and positive Δ). No selection (horizontal line o).



## *1-2- Apostatic selection and polymorphism*

When predators feed on the commoner types in a population then such apostatic selection will favour the rare forms and maintain polymorphism (Clarke, 1962, 1969; Bantock & Harvey, 1974; Bond & Kamil, 2002). Allen & Clarke (1968) were first to test whether wild birds do exert apostatic selection. They presented wild birds with green and brown baits in their surroundings. In one experiment they found that the birds ate more of the prey with which they had previously been conditioned when given an equal proportions of the two prey colours. In the other experiment they found that untrained wild birds tended to collect the common form when presented with the two colours in a 9:1 ratio, although they witnessed a great variation between trials due to behavioural differences among individual birds. However, these two experiments clearly showed that wild birds can select in an apostatic manner. Since then several studies with birds and baits have produced further evidence (Manly *et al.*, 1972; Allen, 1974, 1976; Cook and Miller, 1977; Harvey *et al.*, 1974; Raymond, 1984; Allen *et al.*, 1988).

Clarke (1962) suggested that apostatic selection was the factor responsible for polymorphism in the land snail *Cepaea nemoralis* and *C. hortensis*, based on the negative correlation between the frequency of yellow 'effectively unbanded' shells in the two species collected from mixed colonies around Oxford. In the latest review of *Cepaea*, Cook (1998) agreed with Clarke that from the intensive study of the ecological and morphological of the two snails, it is likely that the polymorphism in *Cepaea* has, in part, been maintained by their predators; for example, thrushes feeding in a frequency-dependent selection way. Similar explanations have been proposed for many other polymorphic species. Examples are the polymorphisms, in the marine snails, *Donacilla cornea* (Whiteley *et al.*, 1997), the blood python *Python brongersmai* (Shine & Harlow, 1998), the guppy fish *Poecilla reliculata* (Endler, 1978) and the spittlebug *Philaenus spumaris* (Owen, 1963).

It has also been suggested that such polymorphism may reduce the proportion of a population taken by predators (Moment, 1962, Croze, 1970; Knill & Allen, 1995). This was tested by Croze (1970) by using crows as predators and polymorphic populations of painted mussels shells with meat rewards underneath. The crows took longer to find a fixed number of shells when they were polymorphic than when they were monomorphic. Thus the polymorphic populations suffered less predation. This might be the case in many of the polymorphic species that live at high densities as well such as the marine bivalve *Donacilla cornea* (Whiteley *et al.*, 1997) and the intertidal snail *Umbonium vestiarium* (Grüneberg, 1980) (which is the subject of chapter 5).

Frequency-dependent selection may also be the factor responsible for the divergence of colour pattern between sympatric species, as a result of indirect interaction between the sympatric species through predators that hunt by sight (Clarke, 1962; Chiba, 1999). This predication was tested by Clarke (1962) using the two nearly identical polymorphic sympatric species *Cepaea nemoralis* and *C. hortensis*. He suggested that if apostatic selection was acting then there would be a negative relationship between frequencies of equivalent morphs of these two species. The results appeared to support the suggestion, but was then criticized by Carter (1967). Chiba (1999) stated that one of the candidates for the divergence of the species of the land snail *Mandarine* could be attributed to a bird *Turdus dauma* which had been introduced to the island where they lived. From studying the relationship between morphology and ecology in eight sister species, he realised that three species (*M. pondersoa*, *M. conus* and *M. mandarina*) that preferred unexposed microhabitats were monomorphic. The sympatric species (*M. aureola*, *M. polita*, *M. hahajimana* and *M. anijimana*) that preferred exposed microhabitats were polymorphic. He suggested that since the latter group have a greater chance of being found by predators, frequency-dependent selection may have produced the polymorphism.

## *1-3- Anti-apostatic selection*

So far we have discussed selection against the common morph, which is called 'apostatic selection' (Clarke, 1962). As the term 'apostate' simply refers to a rare form, Greenwood (1984) argued that since apostatic selection leads to an advantage to rare forms then it should be called 'pro-apostatic selection'. However, predators sometimes develop a preference for the rare morph; in other words, prey items that occur at a low frequency and thus sometimes are described as 'unusual', 'uncommon' or 'odd' (Mueller, 1972). This kind of selection is now called 'anti-apostatic selection' (Greenwood, 1984) and has been particularly observed at a high density (Ohguchi, 1978, 1981; Allen & Anderson, 1984; Greenwood *et al.*, 1984; Allen *et al.*, 1998; Weale *et al.*, 2000). For example Horsley *et al.* (1979) used green and brown pastry baits at a density so high that baits were touching one another. In twelve experiments at different sites selection against brown was stronger when brown was rare than when common.

Unlike pro-apostatic selection which leads to polymorphism (Allen, 1976, 1988b; Chesson, 1984; Clarke, 1962, 1975; Greenwood, 1984, 1985), selection against rare forms leads to monomorphism (Benson, 1972; Edmunds, 1974; Allen, 1988a). Greenwood (1984) stated that pro-apostatic selection is likely to happen when there is a cost of taking mixed diet, when the optimal speed is different for different prey, or when the predator needs to sample its prey to determine its value. On the other hand, anti-apostatic selection is likely when there is a benefit for taking mixed diets, when the rare forms of the prey are easier to detect or pick up, or when the prey are noxious to eat but have to be sampled for this to be determined.

Focusing on one or a few morphologically distinctive (odd) individuals in a prey group may reduce a predator's sensory confusion and thus improve its capture success (Greenwood, 1984; Guilford, 1985). Selection would be anti-apostatic. For example, adult wildebeest that had their horns painted white were found to be exceptionally vulnerable to lions (Kruuk, 1972) and dyed fish were eaten promptly after being returned to a school in the wild (Hobson, 1968). It may be also easier to pick out a single rare form than a single common form because it looks conspicuous standing out against the background of the common one (Allen, 1972; Horsley *et al.*, 1979; Allen & Anderson, 1984; Greenwood, 1984, 1985).

Allen & Anderson (1984) concluded that sampling constant numbers of the available food types will have a disproportionate effect on whichever food is rare, resulting in anti-apostatic selection at the level of the predator population rather than the individual. If there is a sampling then it is predicted that selection will be more anti-apostatic at the start of the feeding trial than towards the end (Allen & Anderson, 1984; Greenwood *et al.*, 1989). Frequency-independent selection among individual birds can be a possible cause of anti-apostatic selection at the population level if different individual predators prefer different colours within a prey population (Allen & Anderson, 1984; Chesson, 1984; Sherratt & MacDougall, 1995). Allen & Anderson (1984) found that while observing three blackbirds in a large outdoor aviary, each bird would tend to eat one colour alone regardless of whether it was common or rare and this single mindedness tended to vary between individuals and between days within individuals. This behaviour would result in anti-apostatic selection overall. However, there is a lack of data on individual predators and thus experiments on individual birds feeding on distasteful prey would shed light on whether anti-apostatic selection, if it takes place, is generated by variation in preferences of individual predators.

Another way of generating anti-apostatic selection is when there is mimicry involved. In experiments with artificial prey and wild birds, O'Donald & Pilecki (1970) found the rare mimic lost its selective advantage when the model was made highly unpalatable. This is because the higher the distastefulness of the model the stronger the reluctance to eat the common mimic. This thus incurs a significant predation on the rare mimic that was overlooked before.

Anti-apostatic selection has been observed in other animals beside birds. Experiments by Ohguchi (1978, 1981) on sticklebacks *Gasterosteus aculeatus* feeding on water fleas *Daphnia magna* produced anti-apostatic selection. Greenwood *et al.* (1984a, 1984b) found that mice *Mus domesticus* preferred the rare form when they were presented with rat cake flavoured vanilla or peppermint and in one experiment with no flavour, and dyed green or brown. Furthermore, Partridge (1981) found that rodents develop increased preferences for temporally rare foods when confined to nutritionally incomplete background diet. Turning to herbivorous insects, Chandra & Wiliams (1983) found that in two plant species, wheat *Triticum vulgare* and chives *Allium schoenoprasum*, the desert locust *Schistocerca gregaria* grazed selectively on the less-favoured plant when it was relatively less abundant.

## *1-4- Factors that affect frequency-dependent selection by predators: density, crypsis and dispersion*

It has been suggested that the strength and the direction of frequency-dependent selection may be affected by many different variables (Clarke, 1962). Such factors are coloration, prey density, dispersion and palatability. Most workers have agreed that these factors will indeed affect both the direction and the strength of the frequency-dependent selection (Allen & Clarke, 1968; Allen, 1972, 1988a; Greenwood, 1984).

### 1-4-1- Prey density

Theory suggests that the strength of frequency-dependent selection by predators should itself depend on the total density of the prey (Clarke, 1962; Greenwood, 1969) and this has been shown in most experiments which have been done in this area. Clarke (1962) predicted that at a very low density selection would be random; predators would tend not to have a preference for a given prey over another because they encounter them so rarely. This persuaded several workers (Greenwood, 1969; Allen, 1972, Cook & Miller, 1977; Horsley *et al.*, 1979; Willis *et al.*, 1980; Allen & Anderson, 1984; Allen *et al.*, 1998; Weale *et al.*, 2000) to investigate the effects of prey density on predator's selection. The general finding from these studies is that at low and intermediate densities selection tends to be apostatic whereas at a high density it changes to anti-apostatic.

Allen (1972) was first to notice that at very high densities selection was anti-apostatic. He presented wild blackbirds with green and brown in two different densities, low and high, and two frequencies, 90% brown and 10% green, and 10% brown and 90% green. At the low density selection was apostatic and at the high density, selection was against the rare form, which he then called "stabilising selection". Harvey *et al.* (1974) criticised Allen's work for placing the two sieves that contained the two different frequencies one metre apart. They argued that the same bird that took the colour which was common in one sieve might continue to take the same colour when rare in the other sieve. Therefore, they repeated the same high density experiment but this time with a distance of 2000 m between the two sieves. Their results showed that the blackbirds took an excess of the common form and thus selected in an apostatic manner. However, since then several experiments have confirmed the results of Allen (1972): (Horsley *et al.*, 1979; Ohguchi, 1981; Allen & Anderson, 1984; Allen *et al.*, 1998; Weale *et al.*, 2000).

Cook and Miller (1977) studied the changing response of the Japanese quails, *Coturnix coturnix japonica* over a range of different densities (1.25 $m^{-2}$, 2.5 $m^{-2}$ and 7.5 $m^{-2}$) by presenting them with five different frequencies of red and blue baits at three different densities. As the density increased, the equilibrium frequency of red declined so that at 7.5 $m^{-2}$ there is an absence of frequency-dependent selection. However, their findings did not show the density at which apostatic selection becomes anti-apostatic. Therefore, Allen *et al.* (1998) conducted another experiment to explore the effect of density on the direction of frequency-dependent selection. Wild birds were presented with 200 baits of green and brown with two levels of frequency, 90% brown and 10% brown, at 4 levels of density, 10 $m^{-2}$, 100 $m^{-2}$, 1000 $m^{-2}$ and 10,000 $m^{-2}$. The results demonstrated that at a density above 131 $m^{-2}$ selection changed from apostatic to anti-apostatic. Thus both the strength and direction of frequency-dependent selection changed with density in a gradual way.

Even selection by humans searching for inconspicuous images on a monitor tends to be affected by the density (Tucker & Allen, 1991). In their experiment Tucker & Allen

(1991) presented eight human subjects with two levels of frequency: 50% and 90%, of dimorphic cryptic computer-generated images at five levels of density, 20, 40, 60, 80 and 100 target images per screen. Their results revealed significant effect of density on selection when prey 1 was common but not when the prey were at equal frequencies. Selection decreased with increasing density. A tendency to select rare forms from a high density of food populations may occur in predators other than birds, such as cats, *Felis silvestris catus* (Church *et al*, 1996) and mice *Mus domesticus* (Greenwood *et al.*, 1984a, 1984b). This anti-apostatic by mammals might have been due to variation in preferences by individuals, the benefit of taking a mixed diet or the ease of locating the rare forms because they stand out against the common forms.

Thus we predict polymorphisms in populations when density is low but monomorphisms when density is high. However, there are many prey species that live at high densities and they are still polymorphic. Examples include the North Atlantic brittlestar, *Ophiopholis aculeata* (Moment, 1962) and the marine bivalve, *Donacilla cornea*, which can reach up to 910 bivalves m$^{-2}$ (Whiteley *et al.*, 1997). Some polymorphic species even live at densities exceeding 10,000 m$^{-2}$, e.g. the intertidal snail *Umbonium vestiarium* (Berry, 1987). Possibly, the massive polymorphisms of these species could help protect by restricting their predators' ability to learn that every colour pattern is palatable (Moment, 1962; Allen, 1988b; Knill & Allen, 1995). This is based on grounds that a predator that selected a prey item would find it difficult to encounter other prey with same colour pattern (Moment, 1962), although it is hard to envisage how such massive polymorphism could evolve other than through frequency-dependent selection (Allen, 1988b). Owen (1963, 1965), based on his work on the African snail *Limicolaria martensiana*, has predicted that diversity increases with increasing density. However, Greenwood (1969) has predicted that apostatic selection is only expected at intermediate densities, i.e. prey will be more polymorphic at intermediate densities rather than at low or high densities. Thus *U. vestiarium* was chosen to investigate whether diversity is influenced by anti-apostatic selection at the extreme high densities characteristic of this snail.

Even with experiments that used dimorphic prey, it is not always the case that selection at high densities is anti-apostatic. For example, in most of Murdoch's (1969) experiments on predation of mussels and barnacles by marine snails, selection was either frequency-independent or apostatic (although it should be stressed that the predators do not use the visual cues alone). In experiments where chicks were offered a high density of prey also showed either apostatic selection (Fullick & Greenwood, 1979) or frequency-independent selection (Willis *et al.*, 1980).

### 1-4-2- Crypsis

In every habitat some prey have evolved to match their background so that predators are less able to detect them (Kettlewell, 1955; Edmunds, 1974). A colour pattern is only considered cryptic if the probability of the detection against its background is low (Endler, 1988). Good examples are the green and brown coloration of many grasshoppers and caterpillars, which match their backgrounds of green and brown vegetation respectively (Edmunds, 1974). The mangrove snail *Littorina scabra* changes from monomorphic dark brown on the dark uniform backgrounds to yellowish on the bright mosaic of the foliage (Cook, 1983).

Kettlewell (1955, 1956) concluded that predators become less selective as they become more familiar with the prey. Later, Tinbergen (1960) introduced the 'searching image' hypothesis while studying the regulation of the density of insect larvae by titmice (Paridae). He noticed that there was a time lag of several days before the appearance of the larvae in the birds' food and soon after this the high intake of the newly discovered species was greater than would have been expected from their abundance in the environment. He concluded that birds tend to search at any one time for only a limited number of prey types, focusing on the distinctive features of common forms and he termed this behaviour as "hunting by searching image". The hypothesis implies changes in the predator's performance that tend to increase its ability to detect particular cryptic prey as a result of recent encounters with similar cryptic prey. Furthermore, searching image must be learned from repeated encounters with common form: predators should search only for a single cryptic food type at any given time and ignore any equally cryptic alternatives (Tinbergen, 1960; Langley, 1996). Other workers soon noticed that predators may preferentially take the more common varieties of variable food (Clarke, 1962; Allen & Clarke, 1968; Croze, 1970; Dawkins, 1971a, 1971b; Manly *et al.*, 1972; Mueller, 1972; Reid & Shettleworth, 1992; Bond & Kamil, 2002).

Like oddity and conspicuousness of prey, searching image is important in determining the selection of prey (Mueller, 1971; Guilford & Dawkins, 1987). Most authors assume that predators that exert apostatic selection do so by acquiring searching images more readily for commonly encountered prey rather than rare prey. However, predators sometimes fail to develop a searching image and then frequency-dependent selection is less likely. For example, some marine species such as *Donax variabilis* (Moment, 1962) and *Umbonium vestiarium* (Grüneberg, 1980) are so very variable in their coloration that almost every individual is different. This may reduce the predator's ability to form a searching image (Croze, 1970; Knill & Allen, 1995). Pietrewicz & Kamil (1981) found that blue jays, *Cyanocitta cristata* failed to form searching images when presented with a mixed "diet" of images of two cryptic species of *Catocala* moth after a long exposure to both of them in an operant conditioning box. Other workers have also confirmed that only one searching image is used at a time and that prey types not matching the image go undetected (Reid & Shettleworth, 1992). Thus 'interference' between conflicting prey targets can limit the development of searching images. Similarly, as discussed already, low encounter rates with a single prey type may give the predator little opportunity to learn the cues that separate prey from background (Owen, 1965).

Harvey *et al.* (1974) found when feeding blackbirds *Turdus merula* that the number of baits taken tended to increase and the frequency of the less common morphs taken tended to decrease on successive days. They suggested that this might indicate that the strength of the searching image increases (although it is important to point out that the prey were not cryptic and thus searching images in the strict sense were probably not used). Their conclusion was that the observed selection behaviour by blackbirds could maintain a rare colour morph in a prey population even when at extremely high density. Recent work by Bond & Kamil (2002) using blue jays *Cyanocitta cristata* selecting 'digital' moths on computer monitors confirms that frequency-dependent selection can maintain polymorphism when searching images are acquired more readily for common than rare prey.

In the case of palatable prey it is clear that the frequency-dependent advantage of rare forms is more likely if their colour pattern matches their surroundings, and can be lost or even reversed if this pattern is not appropriate (Cooper, 1984; Endler, 1988; Tucker &

Allen, 1988). Cooper (1984) conducted a series of experiments to investigate the interaction between apostatic selection and a colour match of the morphs with their background components. He presented wild birds with different frequencies of grey and orange pastry prey at three different backgrounds; one 'grey/orange' to provide colour match with the prey and two 'lilac/yellow and green' for control. He witnessed stronger apostatic selection in the grey/orange background which he attributed to the effect of the match between the prey and the background. This may mean that the rare forms are at a selective advantage if they match their background components. However, does this mean that apostatic selection is not predicted when the polymorphic prey are aposematic and distasteful (Gittleman & Harvey, 1980; Goodale & Snedden, 1977)?. We return to this question later.

### 1-4-3- Dispersion

Predators will maximise their success rate if they search for food in places where prey types are more easily available (Stephens & Krebs, 1986). Thus we predict that palatable prey may become widely dispersed and thus foil the attention of predators by ensuring that the encounter rate is as low as possible. For example Kareiva (1986) reported that the predation rate of ladybirds beetles *Coccinella septempunctata* reached its lowest level when its prey, the aphid *Uroleucon nigrotubercubtu* were dispersed over patches of goldenrods *Solidago canadensis*. This argument should also apply to morphs within populations because searching images are less likely to be acquired when the morphs are dispersed (Tinbergen *et al.*, 1967).

Gianino & Jones (1989) showed that when green and brown pastry 'prey' were randomly intermingled at a low density, wild birds took an excess of the commoner form (agreeing with Allen, 1976) but preferred the rare one when they were aggregated together (agreeing with Horsley *et al.*, 1979). At high densities however, there is no effect of prey dispersion upon direction or strength of frequency-dependent predation by wild birds (Church *et al.*, 1997).

Greenwood *et al.* (1989) tested the effect of dispersion on frequency–dependent selection, using distasteful dimorphic prey and wild birds. In half the trials the rare form was dispersed randomly over the common form and in the other half the rare form was placed in a single clump. The results showed that birds selected more of the rare form when it was intermingled with the common form than when it was distributed in a single clump. Therefore, dispersion might enhance monomorphism in distasteful prey. This interaction between dispersion and distastefulness is discussed further in the next section.

## *1-5- Palatability and aposematic coloration*

### 1-5-1- Aposematism

Predators should not waste time on less rewarding food when the most profitable food is readily available (Curio, 1976). A prey is considered unpalatable if predators attack it less frequently once they have learned it has a nasty taste, and is considered palatable if the attack rate is increased when predators become familiar with it (Goodale & Snedden, 1977; Turner & Speed, 2001). For instance, if palatable and unpalatable prey, whether artificial or insects, are presented to birds, there will be an increase in the predation rate on the palatable prey and a decrease on the unpalatable prey (Greenwood, 1984). There is thus a cost to being palatable. In response, prey have evolved different ways of defending themselves (Endler, 1991). Thus at the two ends of spectrum, some species may evolve crypsis (see above) while others display conspicuous coloration which is associated with distastefulness (aposematism) (Benson, 1972; Sillén-Tullberg, 1985a; Gittleman & Harvey, 1980; Hancox & Allen, 1991; Allen & Cooper, 1994; Marples *et al.*, 1994; Joron *et al.*, 1998; Joron *et al.*, 2001). Other defences, such as Batesian mimicry, have also evolved (Edmunds, 1974) but are largely outside the brief of this work.

Historically, Charles Darwin assumed that bright colours could evolve via sexual selection. However, he realised that sexual selection could not account for the conspicuous colour of non-reproductive larvae, e.g. *Pseudosphinx tetrio* hawk moth caterpillars. He then asked Alfred Wallace, who knew about the theory of mimicry based on predator learning suggested by Henry Bates, about this puzzle. Bates proposed that bright colours might be used by caterpillars to advertise their unpalatability to experienced predators.

Poulton (1890) later expanded this idea to include other signals (e.g. smells and sounds) and called it 'aposematism'.

There are a variety of signals that prey animals use to advertise for their unprofitability that are not visual. These signals may include warning sounds (Dunning, 1995; Rowe, 2002), odours (Ford, 1955; Kaye *et al.*, 1989; Rowe & Guilford, 1996, 2001) and electrical signals (Cott, 1940; Edmunds, 1974; Endler, 1986; Guilford & Dawkins, 1991). For example, predators are expected to avoid the stinging bees after hearing their buzzing. The powerful smell of the gregarious caterpillars of the buff tip moth, *Phalera bucephala* is thought to be a defensive measure against predation (Ford, 1955).

### 1-5-2- Evolution of aposematism

Aposematism represents one of the paradoxes of evolution. Imagine a population of a cryptic species that has recently evolved distastefulness (perhaps because of a shift in a host-plant). A new conspicuous mutant arises. How would this mutant spread, given that the chances of it being eaten because it is conspicuous are very high? Gregariousness in larvae of the same brood in a host-plant may suggest that kin selection is responsible for the spread of mutant (Fisher, 1930; Coppinger, 1970; Harvey & Greenwood, 1978; Mallet & Singer, 1987). Although the conspicuous mutant may get eaten, the predator's 'sampling' might benefit the rest of the population if it avoided them subsequently (Guilford, 1988).

Distasteful prey often display bright coloration to advertise their distastefulness to predators and are thus aposematic (Poulton, 1890; Cott, 1940; Edmunds, 1974; Gittleman & Harvey, 1980; Sword, 1999; Lindström, 2001). This is because bright colours enhance the speed at which predators learn to avoid them after short exposure (Gittleman & Harvey, 1980), although the reasons for this are controversial. Sillén-Tullberg (1985a) tested this hypothesis using two forms of the 5th instar larvae of the hemipteran bug, *Lygaeus equestris*, which can be either grey or bright red. 16 hand-raised great tits *Parus major* were presented with red larvae and another 16 were presented with grey larvae. Both forms were presented at a background matching the grey form. As expected, the survival of the aposematic larvae (the red) was higher than that of the cryptic one (the grey). Mappes & Alatalo (1997) used distasteful brown mealworms as a control when testing the effect of novelty and gregariousness in survival of aposematic prey. They found that the distasteful brown mealworms suffered higher predation by great tits *Parus major* in both aggregated and solitary prey than the pink form (novel) and the black and yellow form (traditional colour for warning).

However, a naive predator would find it easy to detect these brightly coloured individuals and then attack them more than cryptic ones (Endler, 1988). So if prey are always killed in an encounter with a predator, the evolution of aposematic coloration in a distasteful species becomes problematic. Rare aposematic forms would presumably be more easily detected in a population of cryptic individuals and so they will be attacked more (Endler, 1993). This might explain why warning coloration is not expected to occur in rare species (Edmunds, 1974) except if they are able to survive the predators' attack. This should mean that for a rare distasteful prey it is beneficial to be cryptic in order to avoid predation rather than to be conspicuous (Edmunds, 1974; Guilford, 1988, Sword, 1999, 2002), or should evolve to mimic another abundant distasteful to gain more protection (Müllerian mimicry) (Joron & Mallet, 1998).

It is suggested that gregariousness (living at high densities) is more common in aposematic species than in palatable ones (Fisher, 1930; Cott, 1940; Edmunds, 1974; Turner, 1975). The probable reason for this aggregation is that the predators would learn to avoid them after several encounters (Edmunds, 1974; Gagliardo & Guilford, 1993; Gamberale & Tullberg, 1996; Hatle & Salazar, 2001). Several workers have tested this hypothesis. For example, Gagliardo & Guilford (1993) presented domestic chicks, *Gallus gallus domesticus*, with artificial palatable green baits and artificial unpalatable yellow baits in two different treatments (single or aggregated). They found that birds learned to discriminate between the distasteful form and the palatable form faster and more strongly when the prey were aggregated rather than they were singly distributed. Gamberale & Tullberg (1996) examined the same hypothesis but using live prey. They presented domestic chicks with aggregations and solitary individuals of the seed bug *Spilostethus panddurus*. The experiment showed that gregariousness can lower the predator's frequency of attack on aposematic insects. Hatle & Salazar (2001) found that the American bullfrogs *Rana catesbeiana* ate aposematic and gregarious prey significantly later than they ate cryptic gregarious prey. This finding indicates that even 'sit-and wait' predators that use the cues of movement and prey size as cues for prey detection are reluctant to attack gregarious aposematic prey. Thus there is good reason to assume that sharing protection between individuals with similar colours contributes greatly to the success of a 'warning colour' gene (Guilford, 1990).

### 1-5-3- Palatability & frequency-dependent selection

As we have seen, selection on edible, non-mimetic species is often frequency-dependent and predators also learn to avoid distasteful prey. However, little is known about the influence of prey palatability on frequency-dependent selection but monomorphism is predicted to evolve if the prey are unpalatable (Benson, 1972; Allen, 1988a). This is because predators are more likely to learn to avoid commonly encountered morphs than they are rare ones (Allen & Cooper, 1988) and thus kill rare forms disproportionately more often than common forms (Fisher, 1930; Mallet & Singer, 1987; Guilford, 1988). This anti-apostatic selection means that polymorphism is not expected in aposematic species (Brown & Benson, 1974; Endler, 1988). Although outside the scope of this thesis, it is surprising that the neotropical butterfly *Heliconius numata* is both polymorphic and aposematic (Sheppard *et al.*, 1985; Joron *et al.*, 2001). Other examples of polymorphic aposematic species are the two-spot ladybird *Adalia bipunctata* (Creed, 1966; Brakefield, 1985), and the widespread tropical butterfly *Danaus chrysippus* (Smith, 1973a, 1973b).

Greenwood *et al.* (1981 and 1989) were first to test the effect of palatability on the strength of frequency-dependent selection in experiments with artificial prey. In one experiment, in 1981, they presented a range of wild birds with red and yellow distasteful pastry at a low density. They set out 20 trials at 20 different sites; 10 trials were 9 yellow:1 red and the other ten were the reverse. The results showed that selection was frequency-independent. These results however are debatable because no palatable prey were used as a control. In another experiment they fed domestic chicks a high density of distasteful green and brown crumbs. In their design, the chicks were allocated to four different blocks, each block was given one of the four (frequency/palatability) combinations, and each combination was replicated 5 times. The chicks unexpectedly selected the food apostatically. In a third experiment, in 1989, again the wild birds were presented with a low density of distasteful red and yellow baits, but this time the experiment was more carefully controlled. In this experiment 40 trials were set out with 10 trials for each (frequency/palatability) combination. Birds fed independent of frequency on the palatable prey but anti-apostatically on the distasteful prey. This finding of Greenwood *et al.* (1989) indicates that palatability does affect the direction of frequency-dependent selection when the distasteful prey are at low densities. Thus confirms the prediction that at low density, predators will encounter the common distasteful morph often enough to learn that it is distasteful and so will take disproportionately more from the rare form (Benson, 1972; Allen, 1988a).

There have been several experiments that studied the correlation between the survival of the mimic and the frequency and the palatability of the model in Batesian mimicry (Brower, 1960; Morrell & Turner, 1970; O'Donald & Pilecki, 1970; Pilecki & O'Donald, 1971; Goodale & Sneddon, 1977; Gibson, 1980; Gordon, 1987; Allen *et al.*, 1993; Lindström *et al.*, 1997). However, the work done by O'Donald & Pilecki (1970), to test whether frequency-dependent selection maintains mimetic polymorphism, is a classic one. Birds disproportionately selected the artificial common mimic when the model was slightly distasteful but the rare mimic lost its advantage when the model was made highly distasteful.

Turning away from birds, an experiment done by Smithson & MacNair (1997a) showed that bumblebees, typical pollinating insects, when offered artificial flowers that differed in colour, forage in a frequency-dependent way. Smithson & MacNair (1996; 1997a; 1997b) found that bumblebees preferred the most common morph when the flower contained sucrose solution rewards, but visited the rare morph more often than expected by chance when sampling flowers that contained no rewards. An extreme interpretation of this is that even in insects the direction of frequency-dependent selection can be influenced by the degree of 'palatability'.

It has been suggested that predators will eat about the same numbers of two or more unpalatable forms before learning that they are distasteful and therefore kill a higher proportion of the rare forms than of the commoner form (Greenwood, 1984; Allen, 1988a). However, Greenwood *et al.* (1981) found this is not always true and there are some circumstances in which selection is only slightly frequency-dependent and others in which the rare forms are at an advantage. Thus there is little empirical evidence on how frequency-dependent selection is imposed on distasteful prey. Greenwood *et al.* (1989) found that at low density, distasteful prey were selected anti-apostatically because, as mentioned above, predators will encounter the common form frequent enough to learn that it is nasty and therefore will shift their attack to the rare form (Allen, 1988a). So what if the density of the distasteful prey is high? It is expected that at high densities of palatable prey selection will be anti-apostatic (Allen, 1972, Horsley *et al.*, 1979; Allen & Anderson, 1984: Greenwood, 1984; Church *et al.*, 1997; Allen *et al.*, 1998; Weale *et al.*, 2000). So will it be even stronger if the prey are distasteful? This question is central to most of the experiments described in this thesis.

One of three predictions is expected when predators encounter a high density of dimorphic distasteful prey. First, the predators may sample the rare form, which stands out conspicuously against the common form, and on finding it distasteful they may shift their attack to the common form. Because the rare form is conspicuous they will learn faster to avoid it. Therefore selection will become apostatic. Second, a high density of distasteful prey may cause the predators to generalize after consuming the conspicuous rare form. Once they sample the first type of food and find it nasty they might reject the other type because it has a similar appearance (Morrell & Turner, 1970; Greenwood, 1984). This would lead to an increase in anti-apostatic selection. The final possibility is that palatability would have no effect on the strength and direction of frequency-dependent selection and it would stay anti-apostatic as expected in a high density of palatable prey.

## *1-6- Using artificial bait*

It is generally assumed that predators behave in the same way whether the prey are real or artificial and whether the predators themselves are captive or free (Clarke, 1962; Murdoch, 1969; Greenwood, 1984, Allen, 1988a). It has been noticed that birds, whether caged or free, tend to approach unfamiliar food items with considerable caution before they decide to attack (Coppinger, 1970; Shettleworth, 1972). Therefore, predator learning and perception might be best tested with artificial prey (Turner, 1971). Most workers (Allen & Clarke, 1968; Allen, 1972; Greenwood, 1984) prefer to use artificial prey made from pastry dough over natural prey to test the selection of wild birds. This is because they can be made in any size shape or colour and a large number of identical shapes can be produced easily (Raymond, 1987). However, using artificial baits could be disadvantageous because wild birds are not accustomed to eating them in the wild and thus are not exposed to a natural situation.

Turner (1961) was first to use artificial pastry prey by making them from flour and lard dough that was coloured with food dye. This technique was soon used in many experiments on frequency-dependent selection (Allen & Clarke, 1968; Allen, 1972, 1974, 1976; Willis *et al.,* 1980; Greenwood *et al.*, 1981, 1989; Allen *et al.*, 1998). In addition, most of the experiments have used baits that looked conspicuous to the human eye (Cook & Miller, 1977; Willis *et al.*, 1980). In fact, the evidence for both apostatic and anti-apostatic has come from using artificial baits (Allen & Clarke, 1968; Allen, 1972). A justification for the use of bitter tasting chemicals to make prey unpalatable is provided by Reichstein *et al.* (1968) who found that calactin, a defensive chemical, found in some Danaiid butterflies, is persistently bitter.

Mealworms, *Tenebrio molitor* or blowfly maggots, *Calliphora vomitoria,* are live prey but also considered artificial because they can be produced in large quantities and can be dyed and made distasteful. Wilson *et al.* (1990) have produced evidence that anti-apostatic selection becomes stronger with the increasing movement of the prey using *C. vomitoria.* Thus using such live prey in experiments that are designed to test for frequency-dependent selection on distasteful prey would compliment other experiments that used pastry baits and, perhaps, could give a better understanding of how frequency-dependent selection acts on distasteful prey.

## *1-7- Why birds?*

Birds are visually discriminating predators which may acquire short-term prey preferences via, for example, searching images (Tinbergen, 1960; Dawkins, 1971a; Bond & Kamil, 2002) and prey exploitation of dense aggregations (Royama, 1970). Birds can also discriminate between different tastes (Warren & Vince, 1963) and can also detect quinine-flavoured baits (Terhune, 1976).

It is a truism that colour polymorphism in non-mimetic prey species, if maintained by predators, can only be maintained if the predators hunt by sight and have colour vision (Clarke, 1962, 1969). The evidence that birds have excellent chromatic vision is overwhelming. Many workers have shown that they discriminate between different colours of baits (Reiskind, 1965; Goodale & Sneddon, 1977). In the past the assumption was the bird's visual sense is not so different to that of a human (Pumphery, 1948; Kettlewell, 1956) but more recent studies have revealed that birds are more sensitive to ultra-violet and can detect natural targets without having to attain the precise fixation that humans require (Blough, 1979; Bowmaker, 1980; Cuthill *et al.*, 2000; Church *at al.*, 2001). Varela *et al.* (1993) found that birds tend to distinguish between wavelengths of different intensity. A quantitative study on pigeons showed that these pigeons can make a variety of colour matches throughout their visible spectrum (Palacios & Varela, 1992).

Birds can even acquire a preference to a colour that differs but slightly to a very similar colour (Croze, 1970; Allen, 1984). Allen (1984) tested this by presenting pastry of similar colours to wild birds. Seven intermediate colours were made by mixing two types of dough (green and brown) and the nine morphs were made based on the level of greenness from "0-8". He trained a group of birds on one colour and then presented them in "1:1" ratio with another similar colour. From the results he concluded that birds can develop a preference for familiar prey that are similar in colour to an alternative, unfamiliar, colour.

Wild and captive birds are expected to behave in a similar way when feeding on dimorphic prey (Greenwood, 1984). Yet little work has been done to investigate whether 'exotic' species behave in a similar way to European garden birds and thus exert frequency-dependent selection on their prey. To test whether frequency-dependent predation is a general phenomenon, it is essential to increase the range of predators tested.

## *1-8- Aims*

The work was divided into two parts. In summary, the main aim of the first part of the work was to investigate the effect of palatability on frequency-dependent selection by wild birds feeding on populations of prey at high density. It is predicted that selection in distasteful prey will be anti-apostatic against the rare form (Benson, 1972; Allen, 1988a). It is also predicated that selection on palatable prey at high density is anti-apostatic (Allen, 1972; Horsley *et al.*, 1979; Allen *et al.*, 1998; Weale *et al.*, 2000). Thus will anti-apostatic selection on palatable prey be even stronger when the same prey are made distasteful? The first part of the work was designed to answer this question paying attention to the objectives outlined below:

a) To test the effect of palatability on frequency-dependent selection by wild birds when the prey are red and yellow, colours often used by aposematic prey.

b) To test the effect of palatability on frequency-dependent selection by wild birds when the prey are green and brown, colours often used by cryptic prey.

c) To test the effect of palatability on frequency-dependent selection by captive exotic birds.

d) To test the effect of palatability on frequency-dependent selection by different species of wild birds and individuals.

e) To test the effect of palatability on frequency-dependent selection when the prey were live maggots (*Calliphora vomitoria*).

The second part of the work was designed to explore the prediction that levels of polymorphism should be low at very high densities, using *Umbonium vestiarium*, a polymorphic marine snail known to live at very high densities, and to test some of the outcomes using pastry prey.

# Chapter 2

## Experiments with palatable and distasteful artificial prey: selection by wild birds.

## *2-1- Selection on red and yellow baits*

### 2-1-1- Introduction

These experiments were designed to investigate the effect of palatability on frequency-dependent selection on red and yellow distasteful prey at a very high density. Red and yellow colours are common in aposematic insects. For example, yellow contributes to the coloration of some morphs of the butterfly, *Heliconius erato* and its caterpillars (Turner, 1975), and stinging wasps of the genus *Vespula* (Miller, 1961). Red is seen in bitter-tasting ladybirds, *Adalia bipunctata* (Creed, 1966) and poisonous coral snakes, *Micrurus* (Edmunds, 1974; Smith, 1975). The nauseous-smelling lycid beetles, *Lycus,* can be either red with black or yellow with black (Edmunds, 1974).

As far as I am aware, red and yellow baits were used together to test for frequency-dependent selection in few experiments (Willis *et al.*, 1980; Greenwood *et al.*, 1981, 1989; Wilson *et al.*, 1990). All these experiments apart from one (Wilson *et al.*, 1990) failed to demonstrate frequency-dependent selection on palatable prey. However, the majority of the work that used red and yellow, whether one of them or both together, was in experiments that dealt with the effect of warning coloration and its relation to gregariousness (Sillén-Tullberg, 1985a, 1985b), innate and learned aversion (Schuler & Hesse, 1985; Roper & Cook, 1989; Roper, 1990; Rowe & Guilford, 1996) and mimicry (Morrell & Turner, 1970; Hancox & Allen, 1991; Allen *et al.*, 1993).

Two designs were used to test the effect of palatability on yellow and red distasteful pastry baits. In one design wild birds were presented with one level of frequency (either 9:1 or 1:9) and two levels of palatability with two different orders (either palatable first or palatable second). The second design was the design that had been used by Allen & Clarke (1968) in the first experiment of this kind designed to test for apostatic selection and then adopted by other authors (Allen, 1972, 1976; Harvey *et al.*, 1974:

Horsley *et al.*, 1979; Allen & Anderson, 1984; Allen *et al.*, 1998). In their experiments wild birds were presented with prey population of one level of frequency (9-1) and then repeated with a population of (1-9). Horsley *et al.* (1979) stated that the design has the probability of making the results reflect more than 50% of general trends, no matter how statistically significant is the variability between the two populations. One might expect that predators might encounter situations represented by the two designs in nature and thus investigating the effect of palatability on frequency-dependent selection in these two ways would determine whether the effect of palatability on frequency-dependent selection, if any, is different in both designs. Both designs were different from the first experiments that tested the effect of palatability on frequency-dependent selection on dimorphic distasteful pastry baits (Greenwood *et al.*, 1981, 1989).

Unlike the experiments of Greenwood *et al.* (1981, 1989) with wild birds, in this experiment each frequency/palatability combination was replicated four times within each site to ensure that the predators gained sufficient experience to make their choices.

## 2-1-2- Experiment 1 (fixed frequency)

### *2-1-2-1- Prey populations*

The baits were made from plain flour and lard in a 3:1 ratio. Edible colouring dye (Pointing Ltd) of 20 ml strawberry red (K6020) or 16 ml egg yellow (K6013) were added to give the required colours. Hue and value/chroma estimates (Munsell Book of Color, 1967) were: red 5R 5/12 and yellow 10YR 8/12. To make the unpalatable baits 2 g of quinine di-hydrochloride powder was added to the dough (750 g of flour and 250 g of lard). The baits were cylindrical in shape with a diameter of 6 mm and around 7 mm in length (for methods of manufacture, see Allen, 1972, 1974 and Allen *et al.*, 1987).

The baits were presented in 9:1 ratios so that a 'red common' population was 180 reds and 20 yellows and a 'red rare' population was 20 reds and 180 yellows. All 200 baits were presented at a density equivalent to 6400 $m^{-2}$ on a circular foil tray of 175 mm diameter. Each tray was pinned onto a bird table to ensure that the wind did not blow it away. The bird tables were made of a piece of plywood (350x350x10mm) nailed onto a wooden pole (1.5 m high) hammered vertically into the soil. Feeding was encouraged by sprinkling bread crumbs around the trays for several days before the start of the experiment proper. Sites with a fast predation rate were visited more than once a day and the baits which had been eaten were replaced. Other sites, where there was low predation, were visited once a day. I aimed to record the data when 20-100 had been eaten (any bait which had been pecked by birds was considered "eaten"). If there was no predation the tray was moved to another site. When the rain was heavy the trays were removed and were replaced when the rain stopped.

To reduce the probability that the same individuals of bird were eating from more than one site, a distance of not less than 400 m between each site was set (see Harvey *et al.*, 1974).

#### 2-1-2-2- Predators

The birds were free ranging wild birds and included blackbirds *Turdus merula*, house sparrows *Passer domesticus*, robins *Erithacus rubecula*, wood pigeons *Columba palumbus*, starlings *Sturnus vulgaris*, great tits *Parus major*, blue tits *P. caeruleus* and magpies *Pica pica.*

#### 2-1-2-3- Method and Design

This experiment was carried out in the Southampton, Winchester and Eastleigh area between 13th January and 26th April 2000. It had a split-plot design with two levels of palatability (palatable and distasteful), two of prey frequency (red common and red rare), two of order of presentation of the two levels at each site (palatable first followed by distasteful and distasteful first followed by palatable) and four trials per combination of frequency and palatability per site (Table 2-1). Thus in total there were four treatments, representing the four possible combinations of frequency and order of presentation: (a) 90% red with palatable first (b) 90% red with palatable second (c) 10% red with palatable first (d) 10% red with palatable second. Each palatability level was presented in four successive trials per site, giving a total of 192 trials for the whole experiment.

**Table 2-1:** The experimental design of experiment 1 (→) refers to 'followed by'.

- 4 treatments
  - -Treatment 1: red rare palatable→red rare distasteful
  - -Treatment 2: red rare distasteful→red rare palatable
  - -Treatment 3: red common palatable→red common distasteful
  - -Treatment 4: red common distasteful→red common palatable

- 6 sites / treatment = 24 sites
- 2 combinations of frequency and palatability/ treatment
- 4 trials/ combination of frequency and palatability = 192 replicates

### *2-1-2-4- Analysis*

Selection against red in each trial was measured by Manly's β coefficient (Manly, 1973, 1974):

$$\beta = \log(R/r) / [\log(R/r) + \log(Y/y)]$$

Where $R$ = number of red presented
$r$ = number of red left (i.e. not eaten)
$Y$ = number of yellow presented
$y$ = number of yellow left

β ranges from 0 to 1.0. Absence of selection is indicated by a β value of 0.5, a value of >0.5 indicates selection against red and a value of <0.5 indicates selection against yellow.

If all the baits of the rare form were eaten then, to avoid having an infinite number, the number of rare prey that had been left was assumed to be 0.1. All β values were arcsine transformed before applying parametric statistical tests, so that they now ranged from 0 to 90, with a value of 45 indicating no selection. It should be mentioned that any value of β (selection against the red in this case) when subtracted from 90 would give the value of β (against the yellow) for one frequency. For example, 90 - β (against red when rare) = β (against yellow when common).

Rate of predation was used for comparison instead of the total number eaten because the time interval of bait exposure was not uniform, given that the baits were left out until approximately 20-100 baits had been taken.

Because trials were repeated within sites and because many of the individuals were the same, then treating trials as independent of each other leads to pseudoreplication (Hurlbert, 1984). This pseudoreplication would lead to an increase of the degrees of freedom and the chances of a type 1 error occurring. To avoid this, the data were analysed by repeated measures analysis. There are two ways of achieving this, one of which is to treat the sites as a random factor nested within frequency and order of presentation by using MINITAB (general linear model). The total degrees of freedom in this case are reduced to 96. However, the other way, which gave similar results, seemed better suited and thus was used for this particular design. Using SPSS (repeated measures) all four trials within half of a 'treatment' were considered one unit because they were temporal, which is realistic because each of the 4 trials will be dependent on the previous one. This brought down the total degrees of freedom to 48. The sites were again nested within frequency and order.

### *2-1-2-5- Results*

#### *Rate of predation*

If the addition of quinine makes the prey distasteful we expect the predation rate on quinine-flavoured baits to be less than that on non-flavoured baits. The rate of predation on the palatable baits (mean ± S.D.: 9.509 ± 0.928) was higher than the predation rate on the distasteful baits (mean ± S.D.: 4.807 ± 0.665). Repeated measures analysis showed a significant effect of palatability ($F_{1,46} = 5.29$, $P<0.05$) (Table 2-2) indicating that the birds did find quinine-flavoured baits distasteful.

**Table 2-2:** Rate of predation (number of baits taken/ hour) (fixed frequency): repeated measures analysis for differences between palatabilities.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1061.26 | 1061.26 | 5.29 | <0.05 |
| Error | 46 | 9234.46 | 200.75 | | |

### *Frequency-dependent selection*

Table 2-3 shows the mean values of β for both levels of palatability when red was common and when red was rare. The values are all below 45 suggesting frequency-independent selection against yellow. Although selection seemed to be in the direction of anti-apostatic (with β values higher when red was rare than when red was common), repeated measures analysis of variance showed no significant effect of frequency as well as no significant effect of palatability on selection, either as a main effect or in its interaction with frequency. Furthermore, there were no main effects of order of presentation, sites, and no other interactions (Table 2-4).

**Table 2-3:** Frequency-dependent selection on red and yellow (fixed frequency): means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare).

| | Red common | Red rare |
|---|---|---|
| Palatable | 33.35 ± 3.67 | 36.91 ± 5.94 |
| Distasteful | 26.32 ± 4.31 | 40.60 ± 4.32 |

### *Frequency-independent selection*

To test whether there was a statistically significant frequency-independent effect of colour (noticed in the analysis above), β values of red rare were first subtracted from 1 so that all the values represent selection on the common colour, either red common or yellow common. Repeated measures analysis was then applied to the arcsine transformed data.

**Table 2-4:** Frequency-dependent selection on red and yellow (fixed frequency): repeated measures analysis for the effects of palatability, frequency and order of presentation on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 88.690 | 88.690 | 0.086 | n.s |
| Freq | 1 | 3318.834 | 3318.834 | 2.74 | n.s |
| Order | 1 | 192.730 | 192.730 | 0.16 | n.s |
| Site(FO) | 20 | 24229.04 | 1211.45 | 1.168 | n.s |
| Pal*Freq | 1 | 1328.468 | 1328.468 | 1.28 | n.s |
| Pal*Order | 1 | 1823.847 | 1823.847 | 1.76 | n.s |
| Freq*Order | 1 | 67.571 | 67.571 | 0.056 | n.s |
| Pal*Freq*Order | 1 | 2132.127 | 2132.127 | 2.056 | n.s |
| Error | 20 | 20742.36 | 1037.12 | | |

Colour did have a significant effect, with a mean β for red of (± S.D.) 29.79 ± 3.07, and for yellow 51.90 ± 3.67 (Table 2-5) ($F_{1,20}$= 19.37, P<0.001 see Table 2-6). Thus the birds selected more yellows than reds irrespective of frequency.

**Table 2-5:** Frequency-independent selection on red and yellow: (fixed frequency) means (± S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow).

| | Red common | Yellow common |
|---|---|---|
| Palatable | 33.10 ± 3.71 | 53.85 ± 5.99 |
| Distasteful | 26.48 ± 4.87 | 49.95 ± 4.44 |

**Table 2-6:** Frequency-independent selection on red and yellow (fixed frequency): repeated measures analysis for the effects of palatability, colour and order of presentation on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1328.468 | 1328.468 | 1.28 | n.s |
| Colour | 1 | 23468.529 | 23468.529 | 19.37 | <0.001 |
| Order | 1 | 67.571 | 67.571 | 0.056 | n.s |
| Site (FO) | 20 | 24229.03 | 1211.45 | 1.168 | n.s |
| Pal* Colour | 1 | 88.690 | 88.690 | 0.086 | n.s |
| Pal* Order | 1 | 2132.127 | 2132.127 | 2.06 | n.s |
| Colour* Order | 1 | 192.730 | 192.730 | 0.16 | n.s |
| Pal* Colour* Order | 1 | 1823.847 | 1823.847 | 1.76 | n.s |
| Error | 20 | 20742.37 | 1037.12 | | |

## 2-1-3- Experiment 2 (fixed palatability)

### *2-1-3-1- Prey population*

The prey were the same type of red and yellow pastry baits as used in experiment 1. The baits were again presented in 9:1 ratios but this time a 'red common' population consisted of 270 reds and 30 yellows and 'red rare' population was 30 reds and 270 yellows. Therefore, the 300 baits on a circular aluminium foil tray of 175 mm diameter made the density of this experiment equivalent to 11,161 $m^{-2}$ which was higher than that of experiment 1. This design was the same as that of Cramp (1988) and allowed incorporation of her results.

### *2-1-3-2- Method and design*

Unlike experiment 1, 32 sites were used in this experiment, half of which used palatable baits and half unpalatable. For each level of palatability, half the sites started with red common and were then followed by red rare while the other half started with red rare and were then switched to red common. Therefore, this experiment had two levels of frequency (red common and red rare) in each site, two levels of palatability among sites (palatable and distasteful), and two orders of presentation (red common first followed by red rare, and red rare first followed by red common). There were four treatments, each representing one combination of palatability and one order of presentation. The treatments were as follows: (a) palatable with 90% red first (b) palatable with 90% red second (c) distasteful with 90% red first (d) distasteful with 90% red second. Each frequency was presented in four successive trials per site, giving a total of 256 trials for the whole experiment (Table 2-7).

Since the design was the same and 'time' should not be considered as a factor that should be included in the main analysis, I included a data set of an experiment which was conducted in 1988. Therefore, half of the sites were used between November 1987 and March 1988 and were carried out by Cramp (1988). The rest were used between late February and early May 2001 by undergraduate biology students (under my supervision) of Southampton University and myself.

**Table 2-7:** The experimental design of experiment 2 (→) refers to 'followed by'.

- 4 treatments
  - -Treatment 1: red common palatable→red rare palatable
  - -Treatment 2: red rare palatable→red common palatable
  - -Treatment 3: red common distasteful→red rare distasteful
  - -Treatment 4: red rare distasteful→red common distasteful

- 8 sites / treatment = 32 sites
- 2 combinations of frequency and palatability/ treatment
- 4 trials/ combination of frequency and palatability/site = 256 replicates

### *2-1-3-4- Analysis*

Manly's β coefficient calculated as a measure of selection against red. The obtained β values were then arcsine-transformed for normalization. Repeated measures statistical analysis using SPSS was then applied. Sites this time were nested within palatability and order.

### *2-1-3-5- Results*

#### *Rate of predation*

In this experiment only half of the trials (those of Cramp, 1988) were used to investigate the effect of palatability on the rate of predation because the undergraduate students failed to provide the duration of the trial.

Wild birds found the quinine-flavoured baits highly distasteful; their rate of predation was higher on the palatable baits (mean ± S.D.: 12.91 ± 2.03) than that on the unpalatable ones (mean ± S.D.: 3.62 ± 1.05). Table 2.8 shows that there was a highly significant effect of palatability on the rate of predation (frequency main effect: $F_{1,30} = 13.01$, $P<0.001$).

**Table 2-8:** Rate of predation (number of baits taken/ hour) (fixed palatability): repeated measures analysis for differences between palatabilities.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 2764.190 | 2764.190 | 13.011 | <0.001 |
| Error | 30 | 6373.287 | 212.443 | | |

### *Frequency-dependent selection*

Unlike experiment 1, the wild birds selected the baits in a frequency-dependent manner and it was anti-apostatic. The average β values when red baits were rare were higher than when they were common (Table 2-9). There was a highly significant effect of frequency (frequency main effect: $F_{1,28} = 8.38$, $P<0.01$) (Table 2-10). There was no significant effect of palatability and no significant interaction with frequency. Neither were there significant effects of order of presentation, sites and none of the other interactions were significant (Table 2-10).

**Table 2-9:** Frequency-dependent selection on red and yellow (fixed palatability): means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare).

| | Red common | Red rare |
|---|---|---|
| Palatable | 29.81 ± 2.67 | 42.07 ± 3.05 |
| Distasteful | 31.82 ± 3.34 | 46.58 ± 4.05 |

**Table 2-10:** Frequency-dependent selection on red and yellow (fixed palatability): repeated measures analysis for the effects of palatability, frequency and order of presentation on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 679.135 | 679.135 | 1.45 | n.s |
| Freq | 1 | 11683.952 | 11683.952 | 8.38 | <0.01 |
| Order | 1 | 1845.371 | 1845.371 | 3.93 | n.s |
| Site(FO) | 28 | 13133.96 | 469.07 | 0.07 | n.s |
| Pal*Freq | 1 | 99.631 | 99.631 | 0.007 | n.s |
| Pal*Order | 1 | 3.726 | 3.726 | 0.34 | n.s |
| Freq*Order | 1 | 1.255 | 1.255 | 0.00 | n.s |
| Pal*Freq*Order | 1 | 32.146 | 32.146 | 0.023 | n.s |
| Error | 28 | 27235.358 | 972.69 | | |

### *Frequency-independent selection*

There was frequency-independent selection. Like experiment 1, the mean transformed $\beta$ was higher when the colour was yellow than when it was red (mean ± S.D.: 45.68 ± 2.53 for yellow against 30.82 ± 2.11 for red) (Table 2-11). Apart from the main effect of colour ($F_{1,28} = 30.13$, $P<0.01$) the only other significant source of variation was that of site (site main effect: $F_{28,28} = 2.07$, $P<0.05$) (Table 2-12).

**Table 2-11:** Frequency-independent selection on red and yellow (fixed palatability): means (± S.D.) of transformed $\beta$ for both palatabilities (palatable and distasteful) on both colours (red and yellow).

| | Red common | Yellow common |
|---|---|---|
| Palatable | 29.81 ± 2.67 | 47.93 ± 3.05 |
| Distasteful | 31.82 ± 3.34 | 43.42 ± 4.05 |

**Table 2-12:** Frequency-independent selection on red and yellow (fixed palatability): repeated measures analysis for the effects of palatability, colour and order of presentation on transformed $\beta$ values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 99.631 | 99.631 | 0.102 | n.s |
| Colour | 1 | 14135.321 | 14135.321 | 30.13 | <0.001 |
| Order | 1 | 1.255 | 1.255 | 0.001 | n.s |
| Site(PO) | 28 | 272235.36 | 972.69 | 2.07 | <0.05 |
| Pal*Colour | 1 | 679.135 | 679.135 | 1.45 | n.s |
| Pal*Order | 1 | 32.146 | 32.146 | 0.033 | n.s |
| Colour*Order | 1 | 1845.371 | 1845.371 | 3.934 | n.s |
| Pal*Colour*Order | 1 | 3.726 | 3.726 | 0.008 | n.s |
| Error | 28 | 13133.958 | 469.07 | | |

## *2-2- Selection on green and brown*

### 2-2-1- Introduction

Predator colour preferences can be heritable (Alatalo & Mappes, 1996). Colour biases can also be culturally inherited because, for example, birds do not feed their nestlings with aposematic prey. This might result in a preference for cryptic prey and an avoidance of aposematic prey in fledglings (Edmunds, 1974; Krebs, 1978).

In this experiment I used a similar procedure to that of experiment 1 in this chapter but with different colours: brown and green. Most workers have used the combination of these two colours in their experiments when testing frequency-dependent selection in palatable prey (Harvey *et al.*, 1974; Horsley *et al.*, 1979; Allen & Anderson, 1984; Church *et al.*, 1997; Allen *et al.*, 1998; Weale *et al.*, 2000), especially Allen (1972, 1974, 1976, 1984). Allen (1972) and Horsley *et al.* (1979) used them to produce evidence of anti-apostatic at high densities. In fact Allen & Clarke (1968) used green and brown in the first ever experiment designed to test for apostatic selection using artificial pastry baits. Greenwood *et al.* (1981) also used the same colours to test the effect of palatability on selection by seven days old captive chicks feeding on crumbs.

Green and brown are considered less conspicuous when compared to the colours yellow and red used in experiments 1 and 2 which are considered common colours in aposematic terrestrial species (Manly *et al.*, 1972). Many palatable insects are green or brown because they are cryptic. For example, the new world hawkmoth, *Errinyis ello* has caterpillars whose last three instars are polymorphic; either green, brown or grey. The first form feeds in the morning and rests in the leaves where it its better camouflaged, whereas the other two forms they rest during the day on the trunk and feed at night on the leaves (Curio, 1976). Even the pupae of the distasteful butterflies *Pieris brassicae* and *Danaus chrysippus* are either green or brown depending on their surroundings (Poulton, 1890; Edmunds, 1974). Some distasteful prey exhibits cryptic colours when they are solitary or at low densities; for example, some populations of juvenile locusts, *Schistocerca emarginata*, that feed on the unpalatable plant *Ptelea trifoliata*. These populations exhibit density-dependent coloration: they are green (cryptic) at low densities and yellow-and-black at high densities (Sword, 1999). Thus high densities of distasteful prey that are coloured brown or green are not expected. However, the greenish and brownish distasteful pine sawfly larvae *Neodiprion sertifer* are known to live gregariously on their host plant ,Scots pine *Pinus sylvestris* (Esiner *et al.*, 1974).

Thus the main aim of the experiment was to test whether selection of wild birds on unpalatable baits will be affected by palatability if the prey were green and brown. We will compare the results of this experiment with these of the previous one to explore the differences in using inconspicuous colours might have on the original prediction that selection on distasteful prey is anti-apostatic and leads to monomorphism (Benson, 1972; Greenwood *et al.*, 1989).

## 2-2-2- Prey populations

The prey were again cylindrical baits made of flour and lard (7 mm long x 6 mm wide). Brown pastry was made by adding 16 ml of "Coffee Brown" (K6024) food colouring to 1 kg of dough and green pastry was made by adding 10 ml of "Pea Green" (K6027) food colouring to 1 kg of dough. Hue and value/chroma estimates (Munsell Book of Color, 1967) were: green 7.5GY 7/10 and brown 5YR 6/6.

In June 2000, two prey populations of 200 baits were presented in each site: palatable (9G:1B or 1G:9B) and distasteful (9G:1B or 1G:9B). The 200 prey were again presented in a foil tray 175 mm diameter, prey density equivalent to 6400 $m^{-2}$ on a bird table.

## 2-2-3- Predators

Blackbirds, *Turdus merula*, robins *Erithacus rubecula*, and wood pigeons *Columba palumbus*, were among the birds which were seen taking food from the experimental trays.

### 2-2-4- Methods and Design

The experiment was carried out in the Southampton, Winchester and Eastleigh area between 2nd June and 15th August 2000. Twenty sites of the 24 sites in this experiment had also been used in experiment 1.

The experiment had the same basic design as experiment 1 (Table 2-1) except for the colours. It used 24 sites, two levels of palatability (palatable and distasteful), two prey frequencies (green common and green rare), two orders of presentation of the two level at each site (palatable first followed by distasteful and distasteful first followed by palatable) and four trials per level per site. The treatments were as follows: (a) 90% green and palatable first (b) 90% green and palatable second (c) 10% green and palatable first (d) 10% green and palatable second. Each treatment was replicated in 6 sites. Thus the total number of trials was 192.

### 2-2-5- Analysis

The $\beta$ selection coefficient of Manly (1973, 1974) was used to measure the risk to green prey. Repeated measures analysis was then applied to arcsine transformed values of $\beta$ using SPSS. Sites were nested within frequency and order of presentation.

### 2-2-6- Results

*Rate of predation*

From Table 2-13 it can be seen that birds did find the quinine-flavoured baits distasteful: the predation rate was slightly higher on the palatable baits (mean ± S.D.: 2.155 ± 0.166) than on the distasteful baits (mean ± S.D.: 1.184 ± 0.075), and this effect of palatability was statistically significant ($F_{1,46} = 10.70$, $P<0.01$).

**Table 2-13:** Rate of predation (number of baits taken/hour) on green and brown: repeated measures analysis for differences between palatabilities.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 45.32 | 45.32 | 10.70 | <0.001 |
| Error | 46 | 194.89 | 4.24 | | |

### *Frequency-dependent selection*

The results and analysis on the transformed β values are summarised in Tables 2-14 and 2-15. Selection was anti-apostatic, with higher average β values when green baits were rare (mean ± S.D.: 50.67 ± 4.15) than when they were common (25.02 ± 2-21) (Table 2-14). This effect of frequency was significant ($F_{1,20}$ = 15.37, P<0.001) (Table 2-13). From Table 2-14, the difference between the two values for palatable is almost equal to the difference between the two values for distasteful suggesting that the level of anti-apostatic selection is the same in both of the palatable and distasteful prey. This is confirmed by the absence of a significant frequency and palatability interaction (Table 2-15). There was highly significant heterogeneity among sites ($F_{20,20}$ = 6.89, P<0.001) but no other significant main effects or interactions (Table 2-15).

**Table 2-14:** Frequency-dependent selection on green and brown: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (green common and green rare).

| | Green common | Green rare |
|---|---|---|
| Palatable | 25.54 ± 2.91 | 51.85 ± 5.65 |
| Distasteful | 24.49 ± 3.44 | 49.49 ± 6.32 |

**Table 2-15:** Frequency-dependent selection on green and brown: repeated measures analysis for the effects of palatability, frequency, site and order of presentation on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 139.09 | 139.09 | 0.47 | n.s |
| Freq | 1 | 31601.43 | 31601.43 | 15.37 | <0.01 |
| Order | 1 | 327.42 | 327.42 | 0.16 | n.s |
| Site(FO) | 20 | 41113.07 | 2055.65 | 6.89 | <0.01 |
| Pal*Freq | 1 | 20.52 | 20.52 | 0.07 | n.s |
| Pal*Order | 1 | 117.55 | 117.55 | 0.39 | n.s |
| Freq*Order | 1 | 1183.57 | 1183.57 | 0.58 | n.s |
| Pal*Freq*Order | 1 | 13.70 | 13.70 | 0.05 | n.s |
| Error | 20 | 5964.04 | 298.20 | | |

### *Frequency-independent selection*

Repeated measures analysis showed that colour did have a significant effect on selection. The average β value for brown (mean ± S.D.: 39.33 ± 4.15) was much higher than for green (25.02 ± 2.21) (main effect of colours, $F_{1,20} = 4.79$, $P<0.01$) (Tables 2-16 and 2-17). Apart from birds selecting more browns than greens their preference also varied significantly from site to site ($F_{20,20} = 6.89$, $P<0.001$).

**Table 2-16:** Frequency-independent selection on green and brown: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (green and brown).

| | Green common | Brown common |
|---|---|---|
| Palatable | 25.54 ± 2.91 | 38.15 ± 5.65 |
| Distasteful | 24.49 ± 3.44 | 40.51 ± 6.32 |

**Table 2-17:** Frequency-independent selection on green and brown: repeated measures analysis for the effects of palatability, colour, site and order of presentation on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 20.52 | 20.52 | 0.07 | 0.90 |
| Colour | 1 | 9846.13 | 9846.13 | 4.79 | <0.05 |
| Order | 1 | 1183.57 | 1183.57 | 0.58 | n.s |
| Site(OF) | 20 | 41113.07 | 2055.65 | 6.89 | <0.01 |
| Pal*Colour | 1 | 139.09 | 139.09 | 0.47 | n.s |
| Pal*Order | 1 | 13.70 | 13.70 | 0.05 | n.s |
| Colour*Order | 1 | 327.42 | 327.42 | 0.16 | n.s |
| Pal*Colour*Order | 1 | 117.55 | 117.55 | 0.39 | n.s |
| Error | 20 | 5964.4 | 298.22 | | |

## 2-3- Discussion

Anti-apostatic selection is expected to occur at high densities (Allen, 1972; Ohguchi, 1978, 1981; Allen & Anderson, 1984; Greenwood *et al.*, 1984; Allen *et al.*, 1998; Weale *et al.*, 2000). In two of my experiments (2 and 3), wild birds also fed on the high density of distasteful pastry baits anti-apostatically. However, some previous experiments have shown otherwise. For example, domestic chicks fed apostatically on brown and green crumbs at high density, whether they were palatable (Fullick & Greenwood, 1979) or distasteful (Greenwood *et al.* 1981). In addition, experiments by Willis *et al.* (1980) have demonstrated frequency-independent selection but not frequency-dependent selection. Similarly, the result of experiment 1 showed that selection by wild birds on distasteful prey was independent of frequency. Greenwood *et al.* (1981, 1989) found a similar result when testing the effect of palatability: in their first experiment wild birds fed on the unpalatable baits independently of frequency and in their second experiment they fed on the palatable form independently of frequency.

Before discussing the significance of these results within the context of frequency-dependent selection and frequency-independent selection and the effect of palatability on both types of selection, I should consider a possible methodological objection. One might assume that the birds took the rare form first, because it stands out conspicuously, and were able to associate its colour with distastefulness so that in the final trials they shifted their attack to the other colour. This could then result in cancelling out of frequency-dependent selection if the frequency of the prey population stayed constant over the all the trials, as for example in experiment 1. Or it could result in anti-apostatic selection if the frequency was altered, for example as in experiment 2.

### 2-3-1- Frequency-dependent selection

Palatability definitely influenced predation; in all three experiments the rate of predation on the palatable form was higher than on the distasteful one. However, palatability had no significant effect on selection. This consistent result seems to contradict the finding of Greenwood *et al.* (1989) of a significant effect of palatability on frequency-dependent selection. However, it is likely that the significance of these conclusions may have been affected by the levels of prey density that were used in each of the studies. In their experiment, Greenwood *et al.* used a low density of pastry prey (16 m$^{-2}$) and thus, perhaps, birds fed on the common form first and upon associating it with distastefulness they started feeding on the rare form. In the current experiments the birds might have acted as expected when feeding on prey at high density consuming the rare form first and upon learning that it was distasteful, switching to the common form. However, at high prey densities distasteful prey suffer less from the disadvantages of rarity (Lindström *et al.*, 2001). One would expect that the predator would be able to learn quickly that both forms are distasteful and thus generalize or 'perhaps' prefer one colour over another. Thus the strong preference for yellow in experiment 1 and 2 and for brown in experiment 3 might have been a result of such situation. Furthermore, the absence of a difference in the level of frequency-dependent selection between the palatable and the distasteful prey might be explained by the experimental design of these experiments. Once the palatability of the baits was established it did not matter which colour looked more conspicuous; the birds found all the baits equally conspicuous and thus equally memorable as being distasteful.

Green and brown are colours more associated with cryptic coloration than red and yellow, because they are a closer match to soil and vegetation. In experiment 3 these colours, whether distasteful or palatable, were selected anti-apostatically by birds, a result that is in a agreement with other experiments with the same colours in which all prey were palatable (Allen, 1972, Horsley *et al.*, 1979; Weale *et al.*, 2000). However, this selection seemed considerably stronger than that on red and yellow in the first two experiments, especially experiment 1 which had the same design. This could have been due to green and brown being less effective in accelerating the process of learning by predators than the other yellow and red (Gittleman & Harvey, 1980; Sillén-Tullberg, 1985a; Alatalo & Mappes, 1996; Rowe & Guilford, 1996; Lindström *et al.*, 2001).

The absence of a significant effect of frequency-dependent selection in experiment 1 might have been a result of the methodological objection as mentioned above. Or it could reflect variation in hunger among individual birds taking into account that experiment 1 was conducted during winter while the other two were conducted in spring and summer, although part of experiment 2 was conducted in winter. Greenwood *et al.* (1981) suggested that the probable reason that their chicks failed to select anti-apostatically was hunger. Therefore, a factor like this is likely to average out the whole sample.

A significant effect of site was only observed in experiment 3, despite using 20 sites out the 24 sites that had been used in experiment 1 (where there had been no heterogeneity among sites). This difference could have been due to the effect of using green and brown instead of red and yellow or perhaps because the experiment was conducted at a different time of the year when other foods were abundant. Horsley *et al.* (1979) found similar results and called for replication of experiments of this kind.

## 2-3-2- Frequency-independent selection

The predators showed strong frequency-independent colour preference in both experiments but unlike the preference for red in the wild birds used by Greenwood *et al.* (1981), the birds in both experiments 1 and 2 had an overall preference for yellow. This strong preference for the yellow might be due to two reasons. First, the birds may have had an established aversion to red (Sillén-Tullberg, 1985b; Roper, 1990). For example, Roper (1990) found that domestic chicks *Gallus gallus dometicus*, which had no experience with insects, preferred brown to red on both brown and red backgrounds, indicating that red is a strong deterrent colour. In another experiment set up to study aversion, Roper & Cook (1989) showed that naive chicks preferred yellow to green. Thus the results of these two experiments by Roper (1990) Roper & Cook (1989) may indicate that red is a more aversive colour than yellow. Second, it might perhaps have been due to yellow being more conspicuousness than red. Wilson *et al.* (1990) also found that birds preferred yellow over red.

In experiment 3 the birds preferred brown over green. This preference for brown was also observed by Allen (1972) in his experiment which was the first to record anti-apostatic selection at high densities. He noticed the blackbirds had a tendency to take a relative excess of brown than expected on the basis of random predation. Many other experiments (Allen and Clarke, 1968; Allen, 1974, 1976, 1984; Harvey *et al.*, 1974; Horsley *et al*, 1979; Gianino & Jones, 1989, Allen *et al.*, 1998; Weale *et al.*, 2000) have demonstrated that birds often exhibit a preference for brown. This might be due to browns being more conspicuous than greens (at least to human eye) but other explanations are possible (Allen, 1974).

Only experiment 2 (fixed palatability) and experiment 3 showed significant variability in frequency-independent selection among sites. The absence of any significant variability in experiment 1 may mean that the preference for yellow was almost constant in most of the sites and this could have been a factor in birds failing to apply frequency-dependent selection. Other work has identified significant heterogeneity among sites in overall preferences for brown over green (Horsley *et al.*, 1979; Allen & Anderson, 1984).

### 2-3-3- Summary

In conclusion, I have presented two designs which tend to demonstrate that wild birds select the high density of dimorphic distasteful prey in an anti-apostatic manner, except in one experiment. If the baits were morphs of a real species and living gregariously then natural selection would incur a selective disadvantage on the rare form, the frequency of which should thus decrease in the population and may be eliminated. Thus monomorphism is expected at high density of palatable or distasteful prey.

All three experiments showed that at high density of artificial distasteful prey there was no measurable effect of palatability on frequency-dependent selection, although there was a consistently strong preference for one colour over another; yellow over red and brown over green. The strong preference for yellow in experiment 1 is likely to be one of the reasons for the birds failing to apply frequency-dependent selection.

# Chapter 3

## Experiments with palatable and distasteful artificial prey: selection by captive birds, and known species and individuals of free-ranging wild birds.

## *3-1- Selection by captive birds*

### 3-1-1- Introduction

The work reported in this chapter follows on from that in the previous chapter. Populations of distasteful and palatable artificial prey were presented to bird predators at extremely high density with the aim of measuring the effect of palatability on frequency-dependent selection. Thus the experiment had the same design and methods of experiment 1 in chapter 2 but was performed with captive birds of a variety of species. Captive birds are expected to treat a diet containing two different morphs in a similar way to wild birds (Greenwood, 1984). However, Willis *et al.* (1980) found that the density at which apostatic selection becomes anti-apostatic was higher for captive domestic chicks *Gallus gallus domesticus* than for wild birds. Of course we must be careful of such comparison but the result may indicate that the responding behaviour of captive birds might be different from that of wild birds.

Several workers have used captive birds to test frequency-dependent selection. For example, Cook & Miller (1977) used Japanese quail *Coturnix coturnix* to test the relationship between density and the strength of apostatic selection. Greenwood *et al.* (1981) studied the effect of frequency-dependent selection on distasteful food using captive chicks and found that selection was apostatic and thus opposite to the expectation. The authors suggested that hunger was the probable cause of such selection.

Captive or laboratory-bred birds are known to attack novel food in a very short time ("a matter of minutes" (Marples & Kelly, 2001) compared with wild birds ("weeks or even months" (Marples *et al.*, 1998). This suggests that captive birds should show less hesitation when offered pastry baits. Thus the aim of this experiment was to test whether selection by captive birds on baits at a high density is affected by palatability when tested in a controlled environment. It was not possible to use captive birds of the species used in other work described in this thesis. Instead, I used exotic species held in captivity in Oman, where the experiment was conducted in Al-Rodha captive breeding centre between 21 November and 24 January 2001.

### 3-1-2- Predators

The predators were captive birds of five different exotic species housed in eight outdoor cages. The five species of birds were the ring-necked dove *Streptopelia capicola* (13 birds), blue crowned pigeon *Goura cristata* (12 birds), Fisher's lovebird *Agapornis fisheri* (35 birds), masked lovebird *Agapornis personata* (30 birds) and common partridge *Perdix perdix* (17 birds).

The study focused on the last four species listed above, with two cages for each species. However, four of the cages also contained ring-necked doves. Thus overall, the eight cages were as follows: 2 Fisher's lovebird cages, 2 masked lovebird cages, 2 blue crowned pigeon cages (also containing ring-necked doves) and 2 common partridge cages (also containing ring-necked doves).

The number of individual birds in each cage varied from nine birds in the blue crowned pigeon cage to 20 birds in the masked lovebird cage. Six cages were almost identical in size, 3m × 3m × 2.5m. The other two Fisher's lovebird cages were much smaller, 1m × 1m × 1m.

### 3-1-3- Prey populations

Birds were offered the same type of food as given in experiment 1, red and yellow pastry baits. 200 baits were presented to birds in each cage spread haphazardly in a circular foil tray (17.5 cm diameter) on the ground, giving a density equivalent to $6400m^{-2}$. The baits were presented in 9:1 ratios so that a 'red common' population was 180 reds and 20 yellows and 'red rare' population was 20 reds and 180 yellows.

To avoid the effect of hunger the birds had constant access to bird food supplied daily prior to the beginning of each trial. Water and shelter were freely available at all times.

### 3-1-4- Methods and Design

The method was the same as used in experiment 1 in chapter 2 with two colours (red and yellow) and two levels of frequency (red common and red rare), two levels of palatability (palatable and distasteful) and two levels of presentation (palatable first or distasteful first).

4 cages, each containing a different species, were chosen at random to accommodate one level of frequency while the other 4 accommodated the other level. Orders of presentation were equally distributed among sites at random (Table 3-4).

Like previous experiments each trial was terminated when between 20-100 baits had been taken. The baits were presented to birds at 9:00 am, after 2 hrs of feeding, and removed around 4:00 pm. If by then the trial was not terminated, the tray, with the same baits, was again presented in the morning of the next day.

### 3-1-5- Analysis

Manly's $\beta$ coefficient was calculated as a measure of selection against red. Repeated measures statistical analysis using SPSS was then applied. Sites were nested within palatability and order.

### 3-1-6- Results

#### *Rate of predation*

The rate of predation was higher on the palatable baits (mean ± S.D.: 5.012 ± 0.714) than on the distasteful baits (mean ± S.D.: 2.867 ± 0.422). However, this was statistically insignificant ($F_{1,14} = 3.07$, not significant) (Table 3-1), indicating that captive birds were not hesitant in consuming the quinine-flavoured baits.

**Table 3-1:** Rate of predation (number of baits taken/hour): repeated measures analysis for differences between palatabilities.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 73.57 | 73.57 | 3.07 | n.s |
| Error | 14 | 335.66 | 23.98 | | |

### *Frequency-dependent selection*

The rest of the results and analysis for this experiment are summarised in Tables 3-2 and 3-3. Selection was anti-apostatic, with higher average β values when red baits were rare (mean ± S.D.: 53.27 ± 2.82) than when they were common (37.47 ± 1.58) (frequency main effect: $F_{1,4} = 14.67$, $P<0.01$). Although anti-apostatic selection tended to be stronger when the baits were palatable, there was no significant interaction between palatability and frequency. There was no effect of palatability, sites or order of presentation alone (Table 3-3).

Since some cages had two different species and the number of birds varied from cage to cage, it was impossible to consider the effect of species. However, the mean β values for each level of palatability in each cage (Table 3-4), give us some clues about how the two species of lovebirds selected their baits. Through chance, the two cages containing Fisher's lovebirds had the same order of presentation for the two levels of frequency. Comparing the two cages 1 and 8, the birds showed no selection when the baits were distasteful (β was close to 45 for both red rare and red common) but when they were offered palatable baits their selection became anti-apostatic (β was greater for red rare than red common). Masked lovebirds, which had both orders of presentation, also fed anti-apostatically on the palatable baits (β was greater for red rare than red common) and their selection was more independent of frequency when they were given distasteful baits in the second presentation (β was close to 35 for both red rare and red common). These variations among the means suggest that lovebirds selected in an anti-apostatic manner only when the baits were palatable.

**Table 3-2:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare).

| | Red common | **Red rare** |
|---|---|---|
| Palatable | 38.47 ± 2.52 | 57.41 ± 5.02 |
| Distasteful | 36.46 ± 2.37 | 49.12 ± 5.67 |

**Table 3-3:** Frequency-dependent selection: repeated measures analysis for the effects of palatability, frequency, site and order of presentation on selection (transformed β) by captive birds.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 425.30 | 425.30 | 1.503 | n.s |
| Freq | 1 | 3994.59 | 3994.59 | 14.673 | <0.05 |
| Order | 1 | 63.27 | 63.27 | 0.232 | n.s |
| Site(OF) | 4 | 908.995 | 272.248 | 0.962 | n.s |
| Pal*Freq | 1 | 158.08 | 158.08 | 0.559 | n.s |
| Pal*Order | 1 | 487.13 | 487.13 | 1.722 | n.s |
| Freq*Order | 1 | 358.63 | 358.63 | 1.317 | n.s |
| Pal*Freq*Order | 1 | 375.90 | 375.90 | 1.329 | n.s |
| Error | 4 | 1131.695 | 282.924 | | |

**Table 3-4:** Means of β (± S.D.), a measure of selection against red baits for each cage. LB = lovebird.

| Frequency | Order of presentation | Cage | Palatability | |
|---|---|---|---|---|
| | | | Palatable | Distasteful |
| Red rare | Palatable 2nd | 1- Fisher's LB | 51.05 ± 6.37 | 45.65 ± 5.92 |
| | Palatable 1st | 2- masked LB | 66.73 ± 1.86 | 34.72 ± 16.18 |
| | Palatable 2nd | 3- partridge* | 46.69 ± 1.90 | 56.22 ± 2.32 |
| | Palatable 1st | 4- pigeon* | 65.19 ± 4.32 | 59.87 ± 3.86 |
| Red common | Palatable 1st | 5- partridge* | 41.36 ± 4.11 | 39.42 ± 4.72 |
| | Palatable 2nd | 6- masked LB | 40.62 ± 2.96 | 34.99 ± 2.39 |
| | Palatable 1st | 7- pigeon* | 33.51 ± 2.95 | 30.07 ± 5.23 |
| | Palatable 2nd | 8- Fisher's LB | 35.71 ± 7.75 | 44.02 ± 2.25 |

* also contained ring-necked doves.

### *Frequency-independent selection*

The results for the frequency-independent effect of colour are given in Tables 3-5 and 3-6. Captive birds showed no significant consistent preference towards any of the two colours presented, with the average β value for the red (mean ± S.D.: 37.47 ± 1.58) being almost equal to that for the yellow (36.74 ± 2.82) (colour main effect, $F_{1,4} = 0.03$, not significant). Neither was there any significant difference in preferences among the sites.

**Table 3-5:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow).

| | Red common | Yellow common |
|---|---|---|
| Palatable | 38.47 ± 2.52 | 32.59 ± 5.02 |
| Distasteful | 36.46 ± 2.37 | 40.88 ± 5.67 |

**Table 3-6:** Frequency-independent selection: repeated measures analysis for the effects of palatability, colour, site and order of presentation on selection (transformed β) by captive birds.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 158.08 | 158.08 | 0.559 | n.s |
| Colour | 1 | 8.49 | 8.49 | 0.031 | n.s |
| Order | 1 | 358.63 | 358.63 | 1.317 | n.s |
| Site (FO) | 4 | 908.995 | 272.248 | 0.962 | n.s |
| Pal*Colour | 1 | 425.30 | 425.30 | 1.503 | n.s |
| Pal*Order | 1 | 375.90 | 375.90 | 1.329 | n.s |
| Colour*Order | 1 | 63.27 | 63.27 | 0.232 | n.s |
| Pal*Colour*Order | 1 | 487.13 | 487.13 | 1.722 | n.s |
| Error | 4 | 1131.695 | 282.924 | | |

## *3-2- Selection by known species and individuals of free-ranging wild birds*

### 3-2-1- Introduction

Unlike the previous experiment which dealt with captive birds, this one dealt with wild birds. It used a combination of the two designs presented in chapter 2. The course of predation was recorded by a video camera and thus selection by each species was measured separately. In some cases it was possible to measure selection by individuals.

Lawrence (1985) used a video camera when testing the formation of searching image by birds when hunting for cryptic prey. He witnessed considerable variation between different individual great tits and blackbirds in their ability to find cryptic prey. Dawkins (1971a, 1971b) also found similar variation among domestic chicks in their first encounters with cryptic food. Therefore, predation on a single prey is partly dependent on the conspicuousness of the prey itself and partly on the behaviour of the hunting predator (Endler, 1988). Several workers, e.g. Cook (1962), have argued that the behaviour of a predator towards its prey is determined by the first few items it attacks.

At any one time, individual birds of the same species or different species may differ for a variety of reasons in how well they discriminate between two morphs of one prey species or between two different prey types. In their experiments, Allen & Clarke (1968) obtained separate data on blackbirds *Turdus merula*, song thrushes *T. philomelos*, starlings *Sturnus vulgaris*, dunnocks *Prunella modularis*, and house sparrows *Passer domesticus* selecting brown and green artificial baits which were presented at a density of 2 $m^{-2}$. The data showed that selection by wild passerines at low density was apostatic but, in addition, there were noticeable differences in frequency-dependent and frequency-independent selection by the different species.

Variation in predator behaviour within and among species can, of course, be genetic (Curio, 1976). For example, Fox (1972) found that the individuals in wolf litters differed significantly in their exploratory and fearful behaviour when confronted with novel and/ or moving objects. The highest ranking members of the litter were the most exploratory individuals while the subdominant members were the least exploratory. Environmental conditions can also induce variation. For example, Roper (1990) found that rearing chicks in red cages actually reverses their preferences, causing them to prefer red over their usually preferred brown prey.

One of the few experiments that has examined individual behaviour of birds feeding on a high density of green and brown artificial prey was by Allen and Anderson (1984). Captive blackbirds, *Turdus merula*, overall fed anti-apostatically, probably because each individual bird tended to feed on one particular colour. Each individual's preference also tended to change over the course of the trials. These temporal differences are likely to arise from spatial position and chance (Partridge & Green, 1985). Sherratt & MacDougall (1995) found a remarkable variation in preference among the 15 female individuals of the waterboatman *Notonecta glauca* when fed on two prey species the fresh-water shrimps *Gammarus pulex* and the isopod *Asellus aquaticus*. This variation in preference also resulted in an overall anti-apostatic selection. The work by Allen & Anderson (1984) and Sherratt & MacDougall (1995) suggests that anti-apostatic selection by a population of predators can arise from variation in frequency-independent selection by individuals within the population.

Sherratt & MacDougal (1995) stated that variation in preference of individuals predators for particular types of prey is often enough to incur frequency-dependent selection mainly anti-apostatic. The variation in the preference among species might have a greater effect of anti-apostatic than that variation in preference within species. Thus, it was the aim of this experiment to obtain comparative data from a variety of wild bird species and individuals concerning their response to a high density of distasteful prey. Video equipment is ideal for recording wild birds foraging for their natural prey and enables the observer to note differences between individuals and species.

### 3-2-2- Prey populations

The experiment used the same red and yellow baits used in experiment 1. Four different populations of prey (palatable (9R:1Y), distasteful (9R:1Y), palatable (1R:9Y) and distasteful (1R:9Y)) were presented at each site. However, unlike the previous experiments, in this experiment the 200 baits in each population were put on a square plywood tray (total surface area of 625 $cm^2$) rather than a foil dish, to minimise reflection of sunlight into the video camera (Plates 3-1 and 3-2). This gave a density equivalent to 3200 $m^{-2}$, which is about half the density used in the previous experiments, but still considered to be 'high'.

### 3-2-3- Predators

Many wild birds were spotted feeding from the experimental trays but the most regular visitors, which were studied here, were robins *Erithacus rubecula*, house sparrows *Passer domesticus,* blue tits *Parus caeruleus,* Great tits *P. major*, blackbirds *Turdus merula*, wood pigeons *Columba palumbus* and magpies *Pica pica.*

### 3-2-4- Methods and Design

Winter, when food is scarce, is the best period to be able to record large number of species feeding in house gardens (Lack, 1965). Therefore, the experiment was carried out in Southampton during 5 different periods in 3 successive winters. The gardens around Building 62 (Boldrewood), University of Southampton, were used between 23rd November and 11th December 2000. Two house gardens in Glenwood Avenue and Highfield Road were used between 20th January and 30th March 2001. Two sites in the University Halls, Gleneyre and Montefiore, were used between 27th January and 30th March 2002. The same Halls were used again in the following winter between 29th December and 17th February 2003.

As mentioned above there were 4 combinations of both levels of frequency with two levels of palatability within each site, which yields sixteen levels of order of presentation. However, for a practical reason (difficulty in finding sufficient number of appropriate sites where both the number of wild birds is plentiful and the use of the camera is possible) it was hard to apply all the possible levels of order of presentation. Therefore, after incidentally applying 'red common first followed by red rare' for the first the 3 sites, I decided to keep the same order of frequency in all the four remaining sites. Thus only the level of palatability was randomised.

Each treatment (level of palatability x level of frequency) was repeated twice. Therefore in the seven sites the total number of trials was 48. As in the previous experiments, trials were terminated when between 20-100 baits were taken by all the birds combined. The order of presentation was omitted from the analysis because of the difficulties of establishing a sequence of orders for some species which attended only some of the trials.

A Panasonic camera (lens 75cm, CL8658), connected to a Panasonic VCR, was positioned diagonally facing down at the baits from a top floor of a building nearby each experimental site. The camera was connected to a Sony 14 inch monitor to enable sharp focusing on the 200 baits in the wooden square tray. The filming usually started about 7:00 am and finished around 6:00 pm. If the trial had not been completed by 6:00 pm then the tray and baits were removed and presented again in the next day until 20-100 had been removed.

The video recorder had the capacity to store 24 hrs of filming on to a 3 hrs video tape. However, to ensure identification of birds and the colour of the baits taken when using play-back, this 24 hr mode was not used and the tape was taken out and replaced before reaching its normal 3 hrs limit.

To minimize the number of instances when the birds had their backs to the camera (which would have made identification of eaten baits difficult) the side of the tray that faced the camera was covered with blue 'sellotape' and sprinkled with plain white flour in an attempt to deter birds from this part of the tray. When occasionally birds did have their backs to the camera, the number of eaten baits was calculated by subtracting the known number eaten during other observed visits from the number at the last tape replacement. During rain, the filming was stopped and the baits were removed until the rain ceased.

As mentioned above, the trials were terminated when between 20 to 100 baits had been taken. This sometimes meant that each species ate only a few baits during the course

**Plate 3-1:** Video-capture of male blackbird (Veron) feeding on the baits in site 6 (Gleneyre Halls).

**Plate 3-2:** Video-capture of female blackbird (Veronica) feeding on the baits in site 6 (Gleneyre Halls).

of the trial. Therefore, a different formula (see below) was used to calculate β for each species' selection in a single trial (Manly, 1985). If the number taken by each species was less than three baits per each trial then the data of that specific individual was discarded but included in the estimation of overall selection.

During the course of the experiment I was able to identify four individuals (two robins and a pair of blackbirds). At site 3, Gleneyre Avenue, two robin individuals were identified, one individual through a patch on its head while the other through its tameness and tendency to perch close to the birdtable. I once saw them feeding at the same time which may mean that they were a male and female pair. At site 6, Gleneyre Halls, I fed food scraps to a male (Veron) and female (Veronica) blackbird in their territory for seven months prior to the beginning of the recording. Thus I was able to recognize them from their behaviour while feeding.

## 3-2-5- Analysis

Overall selection was measured by Manly's (1973, 1974) β coefficient against red using the formula used in previous experiments:

$$\beta = \log(R/r) /[ \log(R/r) + \log(Y/y)]$$

Where $R$ = number of red presented
$r$ = number of red left
$Y$ = number of yellow presented
$y$ = number of yellow left

Selection by each species was calculated by summing the numbers of the two colours taken over all the visits for a given treatment of frequency/palatability. Beta was then calculated from these totals assuming that the available baits were in a 9:1 ratio, using the formula (Manly, 1985):

$$\beta = (R_e/R_p) /[(R_e/R_p) + (Y_e/Y_p)]$$

Where $R_e$ = number of red eaten
$R_p$ = number of red presented

$Y_e$ = number of yellow eaten
$Y_p$ = number of yellow presented

The rate of predation of one species was measured by measuring the time between the first visit and the last visit made by that species within one trial. Number of baits taken per visit was also used to test for the effect of palatability.

## 3-2-6- Results

### 3-2-6-1- Overall predation

Only robins, house sparrows, blackbirds and blue tits showed regular interest in the food. Observations suggested that some individuals would continue to choose the same colour and completely ignore the other one, irrespective of rarity. This effect will be examined later. Current focus is on the overall predation of the four species combined.

*Rate of predation*

Despite the higher rate of predation by all birds combined on the palatable baits (mean ± S.D.: 7.78 ± 1.96) over the distasteful baits (mean ± S.D.: 2.87 ± 0.19), statistical analysis showed the difference was not significant (Table 3-7).

**Table 3-7:** Rate of predation (number of baits taken/hour): repeated measures analysis for differences between palatabilities for all species combined.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 494.008 | 494.008 | 3.808 | n.s |
| Error | 26 | 3372.951 | 129.729 | | |

*Frequency-dependent selection*

Repeated measures ANOVA confirmed that overall selection was anti-apostatic, with higher average β values when red baits were rare (mean ± S.D.: 61.89 ± 3.33) than when they were common (25.65 ± 2.01) (frequency main effect: $F_{1,18} = 75.32$, $P<0.01$). Like the experiment 2 in chapter 2, this anti-apostatic selection appeared weaker on distasteful prey but in fact there was no significant effect of palatability and no significant interaction of palatability with frequency (Tables 3-8 and 3-9).

**Table 3-8:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for predation by all species combined.

| | Red common | Red rare |
|---|---|---|
| Palatable | 28.85 ± 3.58 | 68.16 ± 2.36 |
| Distasteful | 22.45 ± 1.83 | 55.61 ± 5.86 |

**Table 3-9:** Frequency-dependent selection: repeated measures analysis for the effects of palatability, frequency and site on the overall selection (transformed β) by all species combined.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1510.547 | 1510.547 | 3.974 | n.s |
| Freq | 1 | 17469.531 | 17469.531 | 45.962 | <0.01 |
| Site | 6 | 1813.396 | 302.233 | 0.795 | n.s |
| Pal*Freq | 1 | 223.442 | 223.442 | 0.588 | n.s |
| Error | 18 | 6841.588 | 380.088 | | |

### *Frequency-independent selection*

Colour had no significant effect on overall selection; the average β value for the red when common (mean ± S.D.: 25.65 ± 2.01) was almost the same as for yellow when common (28.11 ± 3.33) (colour main effect: $F_{1,18} = 0.26$, not significant) (Tables 3-10 and 3-11). Thus red and yellow appeared to be equally preferred.

**Table 3-10:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for all species combined.

| | Red common | Yellow common |
|---|---|---|
| Palatable | 28.85 ± 3.58 | 21.84 ± 2.36 |
| Distasteful | 22.45 ± 1.83 | 34.39 ± 5.86 |

**Table 3-11:** Frequency-independent selection: repeated measures analysis for the effects of colour, palatability and site on the overall selection (transformed β) by all species combined.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 132.967 | 132.967 | 0.401 | n.s |
| Colour | 1 | 84.991 | 84.991 | 0.256 | n.s |
| Site | 6 | 2804.408 | 467.401 | 1.409 | n.s |
| Pal*Colour | 1 | 1256.625 | 1256.625 | 3.789 | n.s |
| Error | 18 | 5969.010 | 331.612 | | |

### *3-2-6-2- Robin predation*

Robins were always the first to feed on the baits in the experimental trials that they attended. They never took more than one colour during the same visit when feeding on distasteful prey.

#### *Rate of predation*

Robins were not hesitant at eating quinine dihydrochloride flavoured baits. The average number of baits taken per hour (rate of predation) were as follows (mean ± S.D.: 4.214 ± 3.289) on palatable and (mean ± S.D.: 0.757 ± 0.106) on distasteful. There was no statistically significant effect of palatability on the rate of predation (Table 3-12).

Yet if predation rate is measured by the number of baits taken per visit there was a statistical difference; robins took more baits per visit when they fed on the palatable baits than when they fed on the distasteful ones ($\chi^2_{(1)} = 9.30$, $P<0.01$) implying that they did find the quinine-flavoured baits distasteful.

**Table 3-12:** Rate of predation (number of baits taken/hour): repeated measures analysis for differences between palatabilities for robins.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 0.402 | 0.402 | 1.068 | n.s |
| Error | 20 | 7.526 | 0.376 | | |

#### *Frequency-dependent selection*

The average transformed β values for red rare (mean ± S.D.: 54.34 ± 4.40) is higher than for red common (23.98 ± 3.96) (main effect of frequency: $F_{1,13} = 11.907$, $P<0.01$) implying that selection was anti-apostatic (Tables 3-13 and 3-14). There was no statistical significant effect of palatability, no significant interaction between palatability and frequency and no significant heterogeneity among sites (Table 3-14).

**Table 3-13:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for robins.

| | Red common | Red rare |
|---|---|---|
| Palatable | 26.48 ± 7.04 | 51.85 ± 8.05 |
| Distasteful | 21.50 ± 3.87 | 57.09 ± 3.11 |

**Table 3-14:** Frequency-dependent selection: repeated measures analysis for the effects of palatability, frequency and site on selection (transformed β) by robins.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 13.400 | 13.400 | 0.018 | n.s |
| Freq | 1 | 8787.655 | 8787.655 | 11.907 | <0.01 |
| Site | 5 | 2301.683 | 460.337 | 0.624 | n.s |
| Pal*Freq | 1 | 406.580 | 406.580 | 0.551 | n.s |
| Error | 13 | 9594.487 | 738.037 | | |

### *Frequency-independent selection*

Table 3-15 shows mean selection against red when common and yellow when common. Although the robins tended to have a higher preference for yellow, this was not statistically significant. There was significant variation between sites, implying that the robins' colour preferences differed from site to site (site main effect: $F_{5,13} = 3.5$, $P<0.05$) (Table 3-16).

**Table 3-15:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for robins.

| | Red common | Yellow common |
|---|---|---|
| Palatable | 26.48 ± 7.04 | 38.15 ± 8.05 |
| Distasteful | 21.49 ± 3.87 | 32.91 ± 3.11 |

**Table 3-16:** Frequency-independent selection: repeated measures analysis for the effects of colour, palatability and site on selection (transformed β) by robins.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 406.580 | 406.580 | 1.042 | n.s |
| Colour | 1 | 1606.993 | 1606.993 | 4.120 | n.s |
| Site | 5 | 6826.002 | 1365.200 | 3.5 | <0.05 |
| Pal*Colour | 1 | 13.400 | 13.400 | 0.034 | n.s |
| Error | 13 | 5070.169 | 390.013 | | |

### *3-2-6-3- Robins: individual predation*

*Frequency-dependent selection*

In one site I managed to measure the predation by two robin individuals (probably a male and female). Tables 3-17 and 3-18 show that both individuals exerted anti-apostatic selection. This is indicated by the significant effect of frequency (frequency main effect: $F_{1,2} = 78.419$, $P<0.05$). Individual B had a stronger preference for the rare form compared to that of individual A (individual main effect: $F_{1,2} = 170.778$, $P<0.05$). There was no effect of palatability on selection.

**Table 3-17:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for two robin individuals.

| | Palatable | | Distasteful | |
|---|---|---|---|---|
| | Red common | Red rare | Red common | Red rare |
| Individual A | 0 | 25.38 ± 25.38 | 6.63 ± 6.63 | 22.50 ± 22.5 |
| Individual B | 34.01 ± 2.69 | 77.79 ± 1.74 | 39.6 ± 14.36 | 62.73 ± 2.03 |

**Table 3-18:** Frequency-dependent selection: repeated measures analysis for the effects of palatability and frequency on selection (transformed β) by two robin individuals.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 8.175 | 8.175 | 0.219 | n.s |
| Freq | 1 | 2924.627 | 2924.627 | 78.419 | <0.05 |
| Individual | 1 | 6369.140 | 6369.140 | 170.778 | <0.01 |
| Pal*Freq | 1 | 227.366 | 227.366 | 6.096 | n.s |
| Freq*Individual | 1 | 164.530 | 164.530 | 4.412 | n.s |
| Error | 2 | 74.590 | 37.295 | | |

*Frequency-independent selection*

From Tables 3-19 and 3-20 it can be seen that both individuals had colour preferences (colour main effect: $F_{1,2} = 55.944$, $P<0.05$). Individual A had a preference for yellow at both level of palatability. Individual B slightly preferred red instead and therefore there was a highly significant interaction of colour with individual ($F_{1,2} = 170.778$, $P<0.05$). The colour of the first bait taken by either individual was always the same in all the eight trials, whether it was the colour of the common form or the colour of the rare form. However, individual A always took yellow first, while individual B always took red first (right hand column, Table 3-21).

**Table 3-19:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for two robin individuals.

| | Palatable | | Distasteful | |
|---|---|---|---|---|
| | Red common | Yellow common | Red common | Yellow common |
| Individual A | 0 | 64.62 ± 25.38 | 6.63 ± 6.63 | 67.5 ± 22.5 |
| Individual B | 34.01 ± 2.69 | 22.21 ± 1.739 | 39.6 ± 14.36 | 17.27 ± 2.03 |

**Table 3-20:** Frequency-independent selection: repeated measures analysis for the effects of colour and palatability on selection (transformed β) by two robin individuals.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 227.366 | 227.366 | 6.096 | n.s |
| Colour | 1 | 2086.437 | 2086.437 | 55.944 | <0.05 |
| Individual | 1 | 164.530 | 164.530 | 4.412 | n.s |
| Pal*Freq | 1 | 8.175 | 8.175 | .219 | n.s |
| Colour*Individual | 1 | 6369.140 | 6369.140 | 170.778 | <0.01 |
| Error | 2 | 74.590 | 37.295 | | |

**Table 3-21:** Data for the two robin individuals A & B: Pal refers to palatability (1= palatable and 2= distasteful). Freq refers to frequency (1= red common and 2= red rare). Y refers to yellow and R refers to red.

| Individual | Red presented | Red eaten | Yellow presented | Yellow eaten | Total eaten | Tran-β | Pal | Freq | First colour taken |
|---|---|---|---|---|---|---|---|---|---|
| A | 180 | 0 | 20 | 11 | 11 | 0 | 2 | 1 | Y |
| A | 180 | 3 | 20 | 6 | 9 | 13.26 | 2 | 1 | Y |
| A | 180 | 0 | 20 | 8 | 8 | 0 | 1 | 1 | Y |
| A | 180 | 0 | 20 | 11 | 11 | 0 | 1 | 1 | Y |
| A | 20 | 2 | 180 | 18 | 20 | 45.00 | 2 | 2 | Y |
| A | 20 | 0 | 180 | 8 | 8 | 0 | 2 | 2 | Y |
| A | 20 | 0 | 180 | 12 | 12 | 0 | 1 | 2 | Y |
| A | 20 | 2 | 180 | 12 | 14 | 50.77 | 1 | 2 | Y |
| B | 180 | 6 | 20 | 3 | 9 | 25.24 | 2 | 1 | R |
| B | 180 | 17 | 20 | 1 | 18 | 53.96 | 2 | 1 | R |
| B | 180 | 10 | 20 | 2 | 12 | 36.70 | 1 | 1 | R |
| B | 180 | 10 | 20 | 3 | 13 | 31.32 | 1 | 1 | R |
| B | 20 | 1 | 180 | 2 | 3 | 64.76 | 2 | 2 | R |
| B | 20 | 6 | 180 | 17 | 23 | 60.70 | 2 | 2 | R |
| B | 20 | 9 | 180 | 5 | 14 | 76.05 | 1 | 2 | R |
| B | 20 | 13 | 180 | 4 | 17 | 79.52 | 1 | 2 | R |

### ***3-2-6-4- Blue tit predation***

*Rate of predation*

Unlike robins, blue tits regarded the quinine dihydrochloride baits as distasteful. Their predation rate on palatable (mean ± S.D.: 0.925 ± 0.124) was more than double that on distasteful (mean ± S.D.: 0.405 ± 0.06). This difference was statistically significant ($F_{1,13} = 18.87$, $P<0.01$) (Table 3-22).

Blue tits tended to take a single bait per visit, irrespective of whether the baits were quinine-treated or not and thus there is no statistical differences between the number of baits taken per visit from the prey when they were distasteful and when they were palatable. Blue tits usually ate the baits out of sight of the observer and thus it is possible that fewer distasteful baits were actually swallowed.

**Table 3-22:** Rate of predation (number of baits/hour): repeated measures analysis for differences between palatabilities for blue tits.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 2.271 | 2.271 | 18.87 | <0.01 |
| Error | 13 | 1.564 | 0.12 | | |

*Frequency-dependent selection*

Blue tits seemed to have exerted selection that was towards anti-apostatic. Thus the average β when red was rare (mean ± S.D.: 61.36 ± 5.32) was higher than when red was common (37.12 ± 7.11). However, due to the large variance, there was no statistically significant effect of frequency (Tables 3-23 and 3-24). The effect of palatability, site and the interaction between palatability and frequency were also not statistically significant.

**Table 3-23:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for blue tits.

| | Red common | Red rare |
|---|---|---|
| Palatable | 38.04 ± 8.96 | 57.84 ± 11.68 |
| Distasteful | 35.97 ± 12.11 | 64.37 ± 2.08 |

**Table 3-24:** Frequency-dependent selection: repeated measures analysis for the effects of palatability, frequency and site on selection (transformed β) by blue tits.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1023.338 | 1023.338 | 0.854 | n.s |
| Freq | 1 | 662.874 | 662.874 | 0.470 | n.s |
| Site | 4 | 4896.048 | 1224.012 | 0.869 | n.s |
| Pal*Freq | 1 | 25.995 | 25.995 | 0.018 | n.s |
| Error | 5 | 7046.081 | 1409.216 | | |

*Frequency-independent selection*

Tables 3-25 and 3-26 shows that blue tits had no colour preferences. There was an insignificant effect of colour (although the huge standard deviation might have contributed to this insignificance). There was no heterogeneity among sites.

**Table 3-25:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for blue tits.

| | Red common | Yellow common |
|---|---|---|
| Palatable | 38.04 ± 8.96 | 32.16 ± 11.68 |
| Distasteful | 35.97 ± 12.11 | 25.63 ± 2.08 |

**Table 3-26:** Frequency-independent selection: repeated measures analysis for the effects of palatability, colour and site on selection (transformed β) by blue tits.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 23.190 | 23.190 | 0.046 | n.s |
| Colour | 1 | 702.508 | 702.508 | 1.386 | n.s |
| Site | 4 | 9408.614 | 2352.154 | 4.642 | n.s |
| Pal*Colour | 1 | 548.739 | 548.739 | 1.083 | n.s |
| Error | 5 | 2533.434 | 506.687 | | |

### *3-2-6-5- House sparrow predation*

*Rate of predation*

Although, in the past many studies with artificial prey have shown that house sparrows tend to forage in groups (Allen & Clarke, 1968; Allen, 1976, Raymond, 1984), in this experiment there was never more than one individual visiting each trial at any one time, perhaps reflecting the recent dramatic decline in house sparrow numbers in Britain (Robinson *et al.*, 2001). The rate of predation on the palatable prey (mean ± S.D.: 1.287 ± 0.191) was not significantly higher than the rate of predation on the distasteful ones (mean ± S.D.: 0.780 ± 0.164) (Table 3-27).

Like blue tits the house sparrows took a single bait per visit at both levels of palatability: there is no statistical differences between the number of baits taken per visit when baits were distasteful and palatable. They also usually flew off with the baits.

**Table 3-27:** Rate of predation (number of baits taken/hour): repeated measures analysis for differences between palatabiiities for house sparrow.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 0.292 | 0.292 | 0.547 | n.s |
| Error | 6 | 3.202 | 0.534 | | |

### *Frequency-dependent selection*

Although house sparrows visited the experimental feeding trays less than the previous two species, their selection, like that of robins, was anti-apostatic on both the palatable and distasteful prey. The average β values for red rare (mean ± S.D.: 60.52 ± 6.89) is higher than when red was common (29.03 ± 5.03) (frequency main effect: $F_{1,2}$ = 212.58, P<0.001) (Tables 3-28 and 3-29). Palatability and site had no significant effect.

**Table 3-28:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for house sparrows.

| | Red common | Red rare |
|---|---|---|
| Palatable | 32.46 ± 8.09 | 68.22 ± 3.50 |
| Distasteful | 23.87 ± 3.29 | 55.35 ± 11.39 |

**Table 3-29:** Frequency-dependent selection: repeated measures analysis for the effects of palatability, frequency and site on selection (transformed β) by house sparrows.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 72.923 | 72.923 | 2.567 | n.s |
| Freq | 1 | 6039.074 | 6039.074 | 212.576 | <0.001 |
| Site | 2 | 472.742 | 236.371 | 8.32 | n.s |
| Pal*Freq | 1 | 20.402 | 20.402 | 0.718 | n.s |
| Error | 2 | 56.818 | 28.409 | | |

*Frequency-independent selection*

Overall, house sparrows seemed to have had no particular preference for either red or yellow (Table 3-30). However, there was significant variation among sites (site main effect: $F_{2,2} = 28.05$, $P<0.05$) suggesting that house sparrows had different preferences at different sites (Table 3-31).

**Table 3-30:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for house sparrows.

| | Red common | Yellow common |
|---|---|---|
| Palatable | 32.46 ± 8.09 | 21.78 ± 3.50 |
| Distasteful | 23.87 ± 3.29 | 34.65 ± 11.39 |

**Table 3-31:** Frequency-independent selection: repeated measures analysis for the effects of palatability, colour and site on selection (transformed β) by house sparrows.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 7.020 | 7.020 | 0.77 | n.s |
| Colour | 1 | 44.691 | 44.691 | 4.904 | n.s |
| Site | 2 | 511.332 | 255.666 | 28.05 | <0.05 |
| Pal*Colour | 1 | 44.517 | 44.517 | 4.884 | n.s |
| Error | 2 | 18.228 | 9.114 | | |

### *3-2-6-6- Blackbird predation*

On many occasions blackbirds attended some of the first trials at a given site before either never reappearing or visiting erratically. In addition, like the blackbirds of Allen & Anderson (1984), they tended to take the same colour per visit irrespective of whether that colour was common or rare.

*Rate of predation*

Like the three other species, the results for blackbirds showed no effect of palatability on the rate of predation. Their average rate of predation, although higher on the palatable baits (mean ± S.D.: 13.25 ± 6.98) than on the distasteful ones (mean ± S.D.: 2.74 ± 1.45), was not significantly different, perhaps due to the variability of data (Table 3-32).

There appears to be a statistical difference between the number of baits taken per visit when baits were distasteful and palatable baits ($\chi^2_{(1)} = 19.70$, $P<0.001$), suggesting that blackbirds did find the quinine-treated baits distasteful.

**Table 3-32:** Rate of predation (number of baits taken/hour): repeated measures analysis for the differences between palatabilities for blackbirds.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1008.422 | 1008.422 | 1.747 | n.s |
| Error | 9 | 5194.205 | 577.134 | | |

### *Frequency-dependent selection*

Despite what seemed to be apostatic selection (high β values when red was common and low β values when red was rare (Table 3-33), there was no statistically significant effect of frequency (Table 3-34). As in the data for blue tits, the data were very variable.

**Table 3-33:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for blackbirds.

| | Red common | Red rare |
|---|---|---|
| Palatable | 50.43 ± 13.26 | 18.13 ± 5.56 |
| Distasteful | 46.70 ± 15.08 | 33.35 ± 16.31 |

**Table 3-34:** Frequency-dependent selection: repeated measures analysis for the effects of palatability, frequency and site on selection (transformed β) by blackbirds.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1254.719 | 1254.719 | 1.216 | n.s |
| Freq | 1 | 862.455 | 862.455 | 0.836 | n.s |
| Site | 2 | 10607.692 | 5303.846 | 5.139 | n.s |
| Pal*Freq | 1 | 264.155 | 264.155 | 0.256 | n.s |
| Error | 5 | 5159.986 | 1031.997 | | |

*Frequency-independent selection*

Although the blackbirds tended to prefer yellow (β tended to be higher when the common colour was yellow rather than red), the difference was not statistically significant (Tables 3-35 and 3-36).

**Table 3-35:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for blackbirds.

| | Red common | Yellow common |
|---|---|---|
| Palatable | 50.43 ± 13.26 | 58.55 ± 5.95 |
| Distasteful | 46.70 ± 15.08 | 56.65 ± 15.34 |

**Table 3-36:** Frequency-independent selection: repeated measures analysis for the effects of palatability, colour and site on selection (transformed β) by blackbirds.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 108.918 | 108.918 | 0.047 | n.s |
| Colour | 1 | 2175.653 | 2175.653 | 0.930 | n.s |
| Site | 2 | 4073.735 | 2036.867 | 0.871 | n.s |
| Pal*Colour | 1 | 3857.805 | 3857.805 | 1.649 | n.s |
| Error | 5 | 11693.944 | 2338.789 | | |

### 3-2-6-7- Blackbirds: individual predation

*Frequency-dependent selection*

When selection by the known pair of blackbirds was measured (Tables 3-37 and 3-38), both individuals exerted frequency-dependent selection (frequency main effect: $F_{1,2} = 25.068$, $P<0.05$). Selection tended to be anti-apostatic, and stronger when the prey were distasteful (palatability main effect: $F_{1,2} = 23.53$, $P<0.05$).

**Table 3-37:** Frequency-dependent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare) for two blackbird individuals.

| | Palatable | | Distasteful | |
|---|---|---|---|---|
| | Red common | Red rare | Red common | Red rare |
| Individual A | 70.64 ± 19.36 | 85.76 ± 4.24 | 31.81 ± 9.60 | 73.51 ± 3.96 |
| Individual B | 71.12 ± 18.88 | 70.76 ± 10.76 | 12.69 ± 1.79 | 70.02 ± 1.54 |

**Table 3-38:** Frequency-dependent selection: repeated measures ANOVA for the effects of palatability and frequency on selection (transformed β) by two blackbird individuals.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 3038.730 | 3038.730 | 23.526 | <0.05 |
| Freq | 1 | 3237.897 | 3237.897 | 25.068 | <0.05 |
| Individual | 1 | 344.816 | 344.816 | 2.670 | n.s |
| Pal*Freq | 1 | 1775.744 | 1775.744 | 13.748 | n.s |
| Freq*Individual | 1 | 0.005 | 0.005 | .000 | n.s |
| Error | 2 | 258.325 | 129.163 | | |

**Table 3-39:** Frequency-independent selection: means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and yellow) for two blackbird individuals.

| | Palatable | | Distasteful | |
|---|---|---|---|---|
| | Red common | Yellow common | Red common | Yellow common |
| Individual A | 70.64 ± 19.36 | 4.24 ± 4.24 | 31.81 ± 9.60 | 16.49 ± 3.96 |
| Individual B | 71.12 ± 18.88 | 19.24 ± 10.76 | 12.69 ± 1.79 | 19.98 ± 1.54 |

### *Frequency-independent selection*

Tables 3-39 and 3-40 show that both individuals had a preference for red (colour main effect: $F_{1,2} = 30.88$, $P<0.05$). This preference for red was significantly higher when the prey were palatable suggesting that palatability had an influence on the individuals' colour preferences ($F_{1,2} = 23.53$, $P<0.01$). This was also reflected in the colour of the first bait taken at the start of each trial. In all the eight trials only once was the first bait taken by each individual yellow and this occurred when the baits were distasteful, perhaps after associating red with distastefulness in previous trials (right hand column, Table 3-41).

**Table 3-40:** Frequency-independent selection: repeated measures analysis for the effects of palatability and colour on selection (transformed β) by two blackbird individuals.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1775.744 | 1775.744 | 13.748 | n.s |
| Colour | 1 | 3988.549 | 3988.549 | 30.880 | <0.05 |
| Individual | 1 | 0.005 | 0.005 | 0.000 | n.s |
| Pal*Colour | 1 | 3038.730 | 3038.730 | 23.526 | <0.05 |
| Colour*Individual | 1 | 344.816 | 344.816 | 2.670 | n.s |
| Error | 2 | 258.325 | 129.163 | | |

**Table 3-41:** Data for the two blackbird individuals A (male) & B (female): Pal refers to palatability (1= palatable and 2= distasteful). Y refers to yellow and R refers to red.

| Individual BB | Red presented | Red eaten | Yellow presented | Yellow eaten | Total eaten | Tran-β | Pal | First colour taken |
|---|---|---|---|---|---|---|---|---|
| A | 180 | 7 | 20 | 1 | 8 | 41.41 | 2 | R |
| A | 180 | 3 | 20 | 2 | 5 | 22.21 | 2 | Y |
| A | 20 | 9 | 180 | 4 | 13 | 77.47 | 2 | R |
| A | 20 | 4 | 180 | 5 | 9 | 69.56 | 2 | R |
| A | 180 | 10 | 20 | 0 | 10 | 90 | 1 | R |
| A | 180 | 14 | 20 | 1 | 15 | 51.28 | 1 | R |
| A | 20 | 10 | 180 | 0 | 10 | 90 | 1 | R |
| A | 20 | 10 | 180 | 2 | 12 | 81.52 | 1 | R |
| B | 180 | 1 | 20 | 3 | 4 | 10.89 | 2 | R |
| B | 180 | 3 | 20 | 5 | 8 | 14.48 | 2 | R |
| B | 20 | 5 | 180 | 7 | 12 | 68.48 | 2 | Y |
| B | 20 | 2 | 180 | 2 | 4 | 71.56 | 2 | R |
| B | 180 | 15 | 20 | 1 | 16 | 52.24 | 1 | R |
| B | 180 | 12 | 20 | 0 | 12 | 90 | 1 | R |
| B | 20 | 3 | 180 | 9 | 12 | 60 | 1 | R |
| B | 20 | 10 | 180 | 2 | 12 | 81.52 | 1 | R |

## 3-3- *Discussion*

### 3-3-1- Predation by captive birds

The birds in Al-Rodha captive centre had spent all their life in captivity and, as far as is known, had never encountered unpalatable food previously. However, the captive birds' rate of predation, unlike the wild birds' rate of predation in the three experiments of chapter 2, was not significantly different when feeding on the palatable baits compared with the quinine-flavoured baits. This might have been due to two reasons: first, birds fed on the baits for the purpose of 'sampling' rather than foraging because there was plenty of alternative nutritional food available to them before commencing the experimental trials. Second, the number of birds within a cage varied from 9 birds in some cages to 20 birds in others. This factor (and others) could have contributed to large differences in the rate of predation among the cages, and hence a large error term.

The results of this experiment demonstrated anti-apostatic selection. This result differed from that of the wild birds in experiment 1, chapter 2, which had the same design and colours. The difference might be due to the fact that the experiment with captive birds was more controlled and probably had a significantly high number of birds attending each trial which may have resulted in variation in selection by different individuals that, overall, selection was anti-apostatic (Allen & Anderson, 1984; Sherratt & McDougall, 1995). Furthermore, the difference might be related to wild birds having great experience with distasteful food. Church *et al.* (1996) tested frequency-dependent selection using captive and free-ranging domestic cats, *Felis silvestris catus*. They found that (inexperienced) captive cats produced a weak anti-apostatic response when they fed on a high density of artificial baits in comparison to (experienced) feral cats. Feral cats had previously encountered a range of variable foods (table/farm scraps and live prey) whereas the captive cats have been reared throughout their lives on cat food. Furthermore, the geographical location of the captive birds experiment differed from that of experiment 1. Church *et al.* (1997) found that the strength of anti-apostatic selection differed significantly between two different locations (Southampton in England and Aljarafe in Spain).

The results do not support the results of the experiment of Greenwood *et al.* (1981) which showed that captive chicks fed apostatically on distasteful breadcrumbs of two colours, green and brown. Instead, the experiment is in agreement with the original prediction that selection on distasteful prey is anti-apostatic and leads to monomorphism (Benson, 1972). However, it is important to point out that this anti-apostatic selection occurred irrespective of whether the prey were distasteful or palatable. Therefore, like the previous experiments with wild birds in chapter 2, this experiment showed that palatability had no effect on frequency-dependent selection.

Referring to the lovebirds' behaviour, there seems to have been some effect of palatability on the direction of selection. Fisher's lovebird when presented with the baits in both combinations (red common palatable second and red rare palatable second) had the tendency to feed anti-apostatically on the palatable form and neutrally on the distasteful form. Selection by both groups in cage 1 and 8 seemed to be affected by palatability. However, the variation within selection by the other groups in the remaining cages might have averaged out any statistically significant effect of palatability. This could have been the reason why, although the number of individuals and species varied from cage to cage, there was no significant effect of cage on selection. Experiment 1 in chapter 2, with the same design and colours, but using wild UK birds, also showed no significant effect of site.

The captive birds showed no colour preferences, in contrast to the wild birds in experiment 1 and 2, chapter 2. This might be because the captive birds were fed on a variety of colours of high-quality nutritional bird food and they were habituated to all the colours. Moreover, having different species within some cages might have had an effect, because some might have had a preference for red while others had a preference for yellow, and the overall outcome was neutral. Others have shown that captive birds can have colour preferences (Manly *et al.*, 1972; Willis *et al.*, 1980; Greenwood *et al.*, 1981) but the birds had been brought from hatcheries and thus had had little experience with food items of various colours.

### 3-3-2- Predation of known wild birds species and individuals

Like captive birds, the result for all species combined showed that although they took significantly more palatable baits per unit time than distasteful per unit time, there was no statistical significant effect of palatability. The same was observed in the predation of robins and blackbirds when investigated separately. Only the result for blue tits showed a statistically significant effect of palatability on predation. The insignificance of the effect of palatability on the rate of predation might have been due to the high variability among trials. Each trial was terminated when between 20-100 baits were taken, with an average of (37.8 ± 3.17) in this experiment. In most of the trials, there were a number of different birds species apart from the target species visiting the site, to the extent that sometimes the trial with palatable baits was terminated before any of the target species was observed. Furthermore, magpies and wood pigeons were also among the visitors, and when they were at the vicinity small birds might have deserted the trial until the larger birds had left. This would have resulted in a long time lag in the consumption of the palatable baits by the target species and thus given a predation rate nearer to that of the distasteful baits. Also, because food is usually scarce in the winter, robins, for example, will sample almost anything (even sulphate of potash used for spraying plants, Lack, 1965).

Analysis of the number of baits taken per visit has shown that at least two species, robin and blackbird, did find the quinine-treated baits distasteful. Their consumption per visit was higher on the palatable baits than on the distasteful baits. The other two species, house sparrow and blue tit, took one single bait per visit implying that there was no difference between the two types of prey. However, both those two species usually ate the baits out of sight so it is possible they did not swallow the distasteful baits. In addition, we know from the previous paragraph that the rate of predation by blue tits was affected by palatability. Thus palatability seemed to have affected the predation of all the target species.

Overall, selection measured from the pooled predation by all species combined was anti-apostatic at both levels of palatability agreeing with selection of exotic captive birds. This disagrees with the result from experiment 1 in chapter 2, which had a similar design in terms of the order of the two levels of palatability within one site, and showed no frequency-dependent selection. This difference could have been caused by factors already mentioned in experiment 1, chapter 2. Or it could have been due to having a small number of sites or even the fact that this experiment had all the four combinations of palatability with frequency within one site.

Raymond (1987) tested the preference of several species of wild birds to familiar striped and unstriped pastry prey and found inconsistent results when species were tested alone. However, overall, the pooled data for predation by all the species showed they do prefer the familiar colour. Similarly, in this experiment, out of the four studied bird species, only robins and house sparrows exerted frequency-dependent selection, mainly anti-apostatic. This result is in agreement with the results from several studies that tested selection by known species on high densities of dimorphic prey and found it to be anti-apostatic (Pielowski, 1959; Allen, 1972; Ohguchi, 1978. 1981; Allen & Anderson, 1984; Greenwood *et al.*, 1984a, 1984b). On the other hand, the heterogeneity among the results was high and may have masked any significant effect of frequency on selection of the other two studied species, blue tits and blackbirds. This variation between trials may partly have resulted from blackbirds and blue tits having to learn and remember which colour was associated with distastefulness. The direction of their selection was towards anti-apostatic by blue tits and apostatic by blackbirds. Apostatic selection of blackbirds would support the idea of saturation at high densities (Joron *et al.*, 2001) which implies a distasteful prey species has to reach a high enough density that the rare form could be able to saturate the predators. Thus neutrality of the common and rare forms will persist at all frequencies and thus would lead to the maintenance of highly polymorphic distasteful insects such as *Danaus chrysippus* (Smith, 1973b). However, we need more data for blackbirds feeding from such trials.

Like all the previous experiments in this thesis, palatability had no significant effect on the frequency-dependent selection whether for all species combined or for each of the four birds species treated alone.

Turning to frequency-independent selection, in common with the previous experiments that dealt with captive birds in this chapter, the pooled data showed there was no significant colour preference. Several experiments which have studied selection of a single bird species rather than a wide range of species, have shown that these species when studied separately do exhibit colour preferences. For example, Japanese quails *Coturnix coturnix japonica* preferred red over green (Manly *et al.*, 1972) and blackbirds preferred brown over green (Allen & Anderson, 1984). However, in my experiment, the studied species, robins, blue tits, house sparrows and blackbirds all showed no significant preference for either colour. Yet all the species apart from blue tits took less red when prey were distasteful suggesting again that red is more aversive. Only robins and house sparrows, which also exerted a statistical significant effect of frequency, showed colour preferences that varied from site to site.

### *Frequency-dependent and frequency-independent selection by robin individuals*

It is necessary to have a large sample size and as many replicates as possible to be able to draw meaningful conclusions about the behaviour of individuals. However, the use of a small number of subjects seems acceptable for some workers (Croze, 1970; Allen & Anderson, 1984; Bond & Kamil, 2002).

Comparing the two robins, individual B exerted strong anti-apostatic selection. Individual A had a very strong preference for yellow at both ratios and yet its selection was also anti-apostatic; the preference for yellow was stronger when yellow was rare. An experiment by Bond & Kamil (2002) demonstrated that three blue jays *Cyanocitta cristata* varied considerably in their ability to detect cryptic prey but their pooled data demonstrated a significant accuracy in detecting cryptic prey. Allen & Anderson (1984) found a similar result to that of individual A, with three blackbirds selecting one colour irrespective of frequency. However, when the data were pooled the result showed that selection was anti-apostatic. The pooled data of the two individuals in this experiment also illustrated anti-apostatic selection.

### *Frequency-dependent and frequency-independent selection by blackbird individuals*

The experimental design, with distasteful baits presented first, which was used to test the predation of these two individuals of blackbirds was exactly the same as the one used for the two individuals of robins. Like the predation by the two robin individuals, the combined predation of the two blackbirds individuals was disproportionately higher on the rare form. However, this time both individuals exerted strong anti-apostatic selection on the distasteful baits. Yet individual B, the female, failed to exert frequency-dependent selection on the palatable baits, which resulted in a significant effect of palatability. Since the two individuals were a mated pair, this similarity in selection (exerting strong anti-apostatic selection on distasteful prey and preferring red when fed on palatable prey) could have been due to the two birds copying from each other (Murton, 1971). The experiment by Allen & Anderson (1984) did not reveal any strong evidence for copying but that might have been due to all the three birds being captive and moreover, they were not a pair.

Snow (1989) stated that there seems to be an innate tendency for blackbirds to peck at red objects. This preference for red was apparent when the two individuals fed in my experiment for the first time. The colour of first bait taken by both individuals was red (the colour of the common form). Thereafter the distastefulness, which was associated now with red, might have influenced their next choice which became yellow. This may have resulted in the birds taking an excess of the rare form at both ratios. However, when the palatable baits were presented to them they took an excess of their preferred colour "red" irrespective of whether it was the colour of the common form or the colour of the rare form. Thus palatability appeared to have influenced their selection.

### 3-3-3- Summary

The results of these experiments are promising. They showed that captive exotic birds and some species of European passerines behave as expected when fed on a high density of food. Even at an individual level, selection was anti-apostatic. Only blackbirds showed some tendency to feed on the common form but this was not statistically significant. Palatability seemed to have no influence on selection but was only apparent on selection by the blackbird individuals. However, the experiment with known species and individuals clearly needs more replication at different sites and two orders of frequency like experiment 2 in chapter 1.

# Chapter 4

## Experiments with palatable and distasteful dyed maggots: selection by wild birds.

### 4-1- Introduction

All the experiments in chapters 2 and 3 dealt with prey that were made from pastry and showed no consistent effect of palatability on frequency-dependent selection. However, the prey were artificial and predators might react differently to live prey (Greenwood, 1985; Sillén-Tullberg, 1985a; Sword, 1999; Lindström, 2001). Several workers have used live prey; mainly in testing the effect of aposematic coloration and gregariousness (Järve *et al.*, 1981; Sillén-Tullberg *et al.*, 1982; Sillén-Tullberg, 1985a; Roper, 1990; Marples *et al.*, 1994) but very few have tested for frequency-dependent selection.

Live *Cepaea*, the genus of snail that stimulated the idea of apostatic selection, have been used to test the effect of frequency-dependent selection by such workers as Bantock & Bayley (1973), Bantock & Harvey (1974) and Tucker (1991). Bantock & Bayley (1973) and Bantock & Harvey (1974) used artificial population of live *C. nemoralis* and free-ranging songthrushes *Turdus philomelos* as predators. Tucker (1991) directly tested the effect of frequency-dependent selection using by *C. hortensis* as prey and song thrushes. He presented 4 captive thrushes with 30 snails of 9-1 and 1-9 ratios of five-bandeds to unbandeds and found some evidence that the thrushes exerted apostatic selection.

Other experiments have tested the hypothesis that rare or 'odd' prey are at risk in flocks or swamps. Goshawks *Accipiter gentiles* have been observed attacking a high density of flocks of pigeons *Columbus livia* in an anti-apostatic manner (Pielowski, 1959). Experiments by Ohguchi (1978, 1981) on sticklebacks *Gasterosteus aculeatus* feeding on water fleas *Daphnia magna* also produced anti-apostatic selection. Thus these experiments have provided evidence for anti-apostatic selection at high prey density and so are in agreement with the results produced by using artificial prey (Allen, 1972; Greenwood *et al.*, 1984a, 1984b; Allen *et al.*, 1998; Weale *et al.*, 2000).

Frequency-dependent selection has also been tested using distasteful live prey in their natural environment. Benson (1972) worked on *Heliconius erato*, a distasteful Heliconiid butterfly from Costa Rica. He created rare forms by painting the forewing of some individuals red band black to make them all black. He made the controls by painting the already black wing tips black while retaining the red and black pattern. Two measurements of selection were used. One was residence time, the number of days between the first time a butterfly was observed at the roost after marking and the mid point between the last time seen roosting and the first time not observed. The second method was by recording "major wing damage". His results showed that the rare form survived less, from which he concluded that selection on distasteful prey leads to monomorphism. However, Greenwood *et al.* (1989) argued that an alternative explanation is that the all-black pattern was a less efficient signal of aposematism.

Another experiment using a live prey to test the same hypothesis was carried out by Mallet & Barton (1989). They used the same butterfly *H. erato* but this time translocated the butterflies from one part of its geographical range to another. The experimental butterflies were a 'foreign pattern' that was transferred into the range of another subspecies. One control was the same race as the 'native' subspecies, but transferred to the release site from other sites within the range of the subspecies, while the native controls were captured, marked and immediately released at the same site. Using the same measurements of selection of Benson (1972), a comparison was made between the survival of the introduced rare foreign form with the survival of the introduced native. The findings supported anti-apostatic selection. The survival rate of the introduced foreign butterflies was less than the survival rate of the introduced native ones. However, this experiment also was not conclusive because (perhaps not surprisingly) it had only two replicates and in such a natural environment there might have been many variables that would have affected the results. For example, the released form might have been affected by the new surroundings, perhaps flying less, and therefore less likely to be recapture d(Greenwood *et al.*, 1989).

In the real world it is expected that many prey species of prey under attack by predators would move either to flee the area or to hide (Edmunds, 1974). This movement might, perhaps deliberately, result in sensory confusion which would affect the predator's ability of approaching the prey and would be particularly confusing should several prey individuals move at the same time (Driver & Humphries, 1988). When this happens we might expect that predators would concentrate more on rare prey and thus anti-apostatic selection would be the overall result. Indeed there is evidence for this from an experiment with blowfly *Calliphora vomitora* maggots at a high density (Wilson *et al.*, 1990). In their experiment, Wilson *et al.* (1990) presented wild birds with a prey population of 150 *C. vomitoria* maggots, coloured red and yellow and given in two ratios 1:9 and 9:1, on a specially designed bird table. The maggots' speed of movement was controlled by three different levels of temperature generated by a combination of ambient temperature and a candle under the bird table. The results demonstrated anti-apostatic selection at all speeds of the movement and was strongest at the highest speed.

As stated above, it is possible that predators do not treat artificial prey as ' real' live prey (Greenwood, 1985) and thus their behaviour is abnormal. The aim of the experiments described in this chapter was to test whether using live prey would give a clearer effect of palatability on anti-apostatic selection. As in Wilson *et al.* (1990) the live prey were the larvae of the blowfly *C. vomitoria.* The experiments followed the same design of the first two experiments in chapter 2. In other words both fixed-frequency and fixed-palatability designs were used.

## 4-2- Preliminary experiment

Several experiments have effectively made insect larvae distasteful by adulterating them with quinine salt, for example by removing the rear segment mealworms and placing quinine sulphate powder in (Alcock, 1971) or by dipping mealworms into a solution of quinine didydrochloride (Brower, 1960). In the present experiments, the dipping method was chosen because of the large number of prey required for the experiments. However, it was important to find out whether dipping the larvae of *C. vomitoria* into a solution containing quinine dihydrochloride would indeed make them distasteful to birds. Although previous experiments by Brower (1960) to test Batesian mimicry demonstrated that starlings avoided mealworms *Tenebrio molitor* dipped into 20% and 66% concentrations of quinine dihydrochloride-dipped, it was essential to verify that *C. vomitoria* could be made similarly distasteful.

Six preliminary experiments were conducted in Southampton area at six different sites: Boldrewood (BW), the botanical garden (BG), Southampton common (SC), Gleneyre Halls (GH) and two house gardens in Highfield area in Southampton (HG1 and HG2). In each experiment, 300 larvae of two different colours were presented to birds. At three sites, half of the maggots were red and had been dipped into the solution and the rest were bronze and had not been dipped (for more information on colours, concentration of solution and length of dipping, see next section). The treatment was repeated at three other sites but this time with only the bronze maggots had been dipped. Each experimental site was visited every half an hour and the numbers of the two colours remaining were counted to monitor the progress of the experiment. Each experiment was run until all the maggots of one of the colours, whether red or bronze, were taken. A control experiment, in which water was used instead of the quinine dihydrocloride, was carried out with the same design in six sites: in Boldrewood (BW) and on Southampton common at two different locations (SC1 and SC2), Bencraft Hall (BH) and Montifiore Halls (MH1 and MH2).

**Table 4-1:** Number of maggots left in each of the 12 experiments after one colour had been eliminated. * refers to dipped maggots. Abbreviations of sites have been mentioned in the text.

| Solution-dipped | | | Water-dipped | | |
|---|---|---|---|---|---|
| Site | Red maggots | Bronze maggots | Site | Red maggots | Bronze maggots |
| BW | 53* | 0 | SC1 | 20* | 0 |
| BG | 0 | 42* | SC2 | 18 | 0* |
| SC | 48* | 0 | BC | 9* | 0 |
| GH | 0 | 35* | MH1 | 14 | 0* |
| HG1 | 51* | 0 | MH2 | 11* | 0 |
| HG2 | 0 | 44* | BW | 14 | 0* |

| Summary table | | |
|---|---|---|
| | Bronze-eliminated | Red-eliminated |
| Solution-dipped | 3 | 3 |
| Water-dipped | 6 | 0 |

The result was as expected from the hypothesis that the birds treat quinine-treated larvae as distasteful prey. They took all of the undipped maggots whether they were bronze or red in the quinine-treated experiment but only the bronze maggots were all taken in the water-treated experiment (Table 4-1).

## ***4-3- Experiment 1 (fixed palatability)***

This experiment had the same design as experiment 2 in chapter 2 where distasteful prey were presented to birds in a different set of sites from those used for palatable prey.

### 4-3-1- Prey populations

The prey were larvae of blowfly *Calliphora vomitoria* bought from an angling centre (Homestores, Fishing tackle in Swythling) and already dyed red and bronze. Hue and value/chroma estimates (Munsell Book of Color, 1967) were: red 7.5R 5/14 and bronze 5YR 6/12. To make them taste distasteful the maggots were dipped in a quinine di-hydrochloride solution for three minutes and then left in the fridge until being used. The solution was made by mixing 1g of quinine di-hydrochloride powder in 6 ml of distilled water. 300 maggots were presented to birds inside a circular 92.5 mm diameter aluminium foil tray to make a density equivalent to 11,161 $m^{-2}$. Two ratios of maggots were used: 9 red : 1 bronze and 1 red : 9 bronze.

### 4-3-2- Predators

The predators were free ranging wild birds. Among the species seen feeding were blackbirds *Turdus merula*, house sparrows *Passer domesticus*, robins *Erithacus rubecula*, starlings *Sturnus vulgaris*, great tits *Parus major*, blue tits *P. caeruleus* and wood pigeon *Columba palumbus*.

### 4-3-3- Methods

This experiment was carried out between 24th May and 28th June 2001 in the Southampton, Winchester and Eastleigh area. As mentioned before, the design was similar to that of experiment 2 in chapter 2. Each of the sixteen sites had one level of palatability (palatable or distasteful) and two levels of frequency (red common followed by red rare or red rare followed by red common), two orders of presentation (red common first or red rare first) and four trials per level of frequency, giving a total of 128 trials per experiment. Unlike other experiments in which the baits were left unattended, in this experiment I stayed close to the site and observed each trial until terminated. This is because birds attacked the maggots so quickly that sometimes the trial was completed in less than an hour. On sunny days the maggots were moving against each other in circle very quickly, but on cloudy days the movement was reduced to about half. To stop them from crawling out, 'Slippery Stuff' Gel was applied to the surface of the edge of the dish, but on rainy days not even the gel was able to stop them from crawling out. Trials had to be abandoned when it rained heavily.

## 4-3-4- Analysis

Manly's β was calculated as a measure of selection against red. The obtained β values were then arcsine-transformed for normalization. Repeated measures statistical analysis using SPSS was then applied with site nested within palatability and order of presentation.

## 4-3-5- Results

### *Rate of predation*

Confirming the results of the preliminary experiment, the birds clearly found the dipped maggots distasteful. Their predation rate on palatable maggots (mean ±S.D.: 24.330 ± 0.976) was higher than on distasteful maggots (mean ±S.D.: 20.144 ± 0.533). This difference was statistically significant ($F_{1,30} = 7.57$, $P<0.01$, Table 4-2).

**Table 4-2:** Rate of predation (number of maggots taken/hour) (fixed palatability): repeated measures analysis for differences between palatabilities.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 560.71 | 560.71 | 7.57 | <0.01 |
| Error | 30 | 2222.17 | 74.07 | | |

### *Frequency-dependent selection*

The rest of the results and analysis for this experiment are summarised in Tables 4-3, 4-4 and 4-5. Selection was anti-apostatic, with higher average β values when red maggots were rare (mean ±S.D.: 59.21 ± 0.76) than when they were common (26.81 ± 0.87) (frequency main effect: $F_{1,12} = 1906.77$, $P<0.001$). With a higher difference between the two β values, distasteful maggots were thus selected more anti-apostatically than palatable maggots. This interaction of palatability with frequency was statistically significant ($F_{1,12} = 12.498$, $P<0.01$). This may indicate, unlike the artificial baits experiments, that in this experiment palatability did influence frequency-dependent selection.

From the 'difference in means' columns in table 4-4 it can be seen that there was a tendency for selection on palatable prey to be more anti-apostatic when red common was first. When the prey were distasteful there was stronger anti-apostatic selection when red common was presented second. This is supported by the significant statistical interaction of palatability, frequency and order ($F_{1,12} = 17.802$, $P<0.01$). There was a significant variation among sites (site main effect: $F_{12,12} = 4.192$, $P<0.01$). None of the other interactions were significant.

**Table 4-3:** Frequency-dependent selection (fixed palatability): means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) at both frequencies (red common and red rare).

| | Red common | Red rare |
|---|---|---|
| Palatable | 29.17 ± 1.57 | 58.94 ± 1.35 |
| Distasteful | 24.45 ± 1.29 | 59.47 ± 0.88 |

**Table 4-4:** Means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) when red was common and when it was rare at two levels of presentation (red common first and red common second). Differ in means = differences in means.

| | Red common 1st | | | Red common 2nd | | |
|---|---|---|---|---|---|---|
| | Red common | Red rare | Differ in means | Red common | Red rare | Differ in means |
| Palatable | 28.62 ± 1.19 | 61.86 ± 1.16 | 33.24 | 30.02 ± 2.04 | 56.03 ± 1.39 | 26.01 |
| Distasteful | 26.11 ± 1.23 | 58.64 ± 1.51 | 32.53 | 22.79 ± 1.39 | 60.30 ± 1.23 | 37.51 |

**Table 4-5:** Frequency-dependent selection (fixed palatability): repeated measure analysis for the effects of palatability, frequency, site and order of presentation for red and bronze maggots on transformed β values.

| Source | dF | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 140.58 | 140.58 | 1.904 | n.s |
| Freq | 1 | 33585.84 | 33585.84 | 1906.77 | <0.001 |
| Order | 1 | 66.82 | 66.82 | 0.905 | n.s |
| Site(PO) | 12 | 886.128 | 73.844 | 4.192 | <0.01 |
| Pal*Freq | 1 | 220.13 | 220.13 | 12.498 | <0.01 |
| Pal*Order | 1 | 12.24 | 12.24 | 0.166 | n.s |
| Freq*Order | 1 | 12.95 | 12.95 | 0.735 | n.s |
| Pal*Freq*Order | 1 | 313.56 | 313.56 | 17.802 | <0.01 |
| Error | 12 | 211.373 | 17.614 | | |

### *Frequency-independent selection*

The results for the frequency-independent effect of colour are given in Tables 4-6 and 4-7. Birds had a significant preference towards bronze: the average β value for red (mean ±S.D.: 26.79 ± 0.87) was lower than that of bronze (30.80 ± 0.76) (main effect of colour: $F_{1,12} = 6.88$, $P<0.05$). When maggots were distasteful both colours were preferred less (palatability main effect: $F_{1,12}= 12.497$, $P<0.01$), in other words, selection against the common colour was less, confirming what we already know: selection was more anti-apostatic. There was also a highly significant interaction of palatability with order ($F_{1,12} = 17.80$, $P<0.01$), reflecting what we already know; strong anti-apostatic selection on palatable prey when red common presented first and strong anti-apostatic selection on distasteful prey when red common presented second.

**Table 4-6:** Frequency-independent selection (fixed palatability): means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and bronze).

| | Red common | Bronze common |
|---|---|---|
| Palatable | 29.17 ± 1.57 | 31.06 ± 1.35 |
| Distasteful | 24.45 ± 1.29 | 30.53 ± 0.88 |

**Table 4-7:** Frequency-independent selection (fixed palatability): repeated measures analysis for the effects of palatability, colour, site and order of presentation for red and bronze maggots on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 220.13 | 220.13 | 12.497 | <0.01 |
| Colour | 1 | 508.17 | 508.17 | 6.88 | <0.05 |
| Order | 1 | 12.95 | 12.95 | 0.175 | n.s |
| Site(PO) | 12 | 211.373 | 17.614 | 0.24 | n.s |
| Pal*Colour | 1 | 140.58 | 140.58 | 1.904 | n.s |
| Pal*Order | 1 | 313.56 | 313.56 | 17.8 | <0.001 |
| Colour*Order | 1 | 66.82 | 66.82 | 0.905 | n.s |
| Pal*Colour*Order | 1 | 12.24 | 12.24 | 0.166 | n.s |
| Error | 12 | 886.128 | 73.844 | | |

## *4-4- Experiment 2: fixed frequency*

This experiment, similar to experiment 1 in chapter 2, was designed to investigate whether altering the level of palatability within a site would show an effect of palatability on frequency-dependent selection.

### 4-4-1- Prey Populations

The prey were again larvae of the blowfly *Calliphora vomitoria* of two colours, bronze and red. As before, to make them distasteful the larvae were dipped in a quinine-treated solution (1 g of quinine di-hydrochloride dissolved in 6 ml of water) for 3 minutes.

### 4-4-2- Predators

The predators were free-ranging wild birds. The species seen eating were blackbirds *Turdus merula*, house sparrows *Passer domesticus*, robins *Erithacus rubecula*, blue tits *Parus caeruleus* and magpies *Pica pica.*

### 4-4-3- Methods and Design

This experiment was carried out between 5th August and 14th September 2001 in the Southampton, Winchester and Eastleigh area. The experiment had a similar design to experiment 1 in chapter 2: a split-plot design with two levels of palatability (palatable and distasteful), two of prey frequency (red common and red rare), two of order of presentation of the two levels at each site (palatable first followed by distasteful and distasteful first followed by palatable) and four trials per level of palatability per site.

Sites were again chosen at random with respect to treatment. Because of practicalities some of the sites used were the same as used in the fixed palatability experiment.

### 4-4-4- Analysis

Selection was measured by Manly's β coefficient against red and the arcsine-transformed β values were analysed by repeated measures analysis using SPSS. Sites were nested within frequency and order of presentation.

### 4-4-5- Results

*Rate of predation*

Palatability had a significant effect on the rate of predation and was higher on palatable (mean ±S.D.: 24.111 ± 1.138) than on distasteful prey (mean ±S.D.: 17.971 ± 0.625) ($F_{1,30} = 12.44$, $P<0.01$) (Table 4-8).

**Table 4-8:** Rate of predation (number of maggots taken/hour) (fixed frequency): repeated measures analysis for differences between palatabilities.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 1206.22 | 1206.22 | 12.44 | <0.01 |
| Error | 30 | 2909.40 | 96.98 | | |

*Frequency-dependent selection*

Frequency had a highly statistically significant effect on selection ($F_{1,12} = 148.61$, $P<0.001$) (Table 4-10). Birds fed anti-apostatically on maggots, with stronger selection (mean ±S.D.: 60.28 ± 1.19) when red maggots were rare compared to weak selection (32.80 ± 1.71) when common (Table 4-9). Table 4-10 also shows that there was a significant interaction between palatability and frequency ($F_{1,12} = 8.07$, $P<0.05$) indicating that selection was different between the two levels of palatability. However, in contradiction to the previous experiment, anti-apostatic selection was stronger when the prey were palatable. This time there was no significant difference among sites and neither were there any significant interactions.

**Table 4-9:** Frequency-dependent selection (fixed frequency): means (±S.D.) of transformed β for both palatabilitiets (palatable and distasteful) at both frequencies (red common and red rare).

| | Red common | Red rare |
|---|---|---|
| Palatable | 29.85 ± 2.67 | 62.00 ± 1.83 |
| Distasteful | 35.74 ± 1.69 | 58.56 ± 1.36 |

**Table 4-10:** Frequency-dependent selection (fixed frequency): repeated measures analysis for the effects of palatability, frequency, site and order of presentation for red and bronze maggots on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 48.22 | 48.22 | 0.558 | n.s |
| Freq | 1 | 24182.95 | 24182.95 | 148.605 | <0.001 |
| Order | 1 | 197.81 | 197.81 | 1.216 | n.s |
| Site(FO) | 12 | 1952.793 | 162.733 | 1.885 | n.s |
| Pal*Freq | 1 | 697.23 | 697.23 | 8.074 | <0.05 |
| Pal*Order | 1 | 14.26 | 14.26 | 0.165 | n.s |
| Freq*Order | 1 | 195.87 | 195.87 | 1.204 | n.s |
| Pal*Freq*Order | 1 | 6.01 | 6.01 | 0.07 | n.s |
| Error | 12 | 1036.197 | 86.35 | | |

### *Frequency-independent selection*

When the effect of colour was investigated by calculating β for red when common and bronze when common, there was no evidence for frequency-independent selection (Table 4-11 and 4-12). Palatability had a significant effect on selection (palatability main effect: $F_{1,12} = 8.07$, $P<0.05$), reflecting the lower β values (i.e. increased anti-apostatic selection) when baits were palatable (Tables 4-11 and 4-12).

**Table 4-11:** Frequency-independent selection (fixed frequency): means (±S.D.) of transformed β for both palatabilities (palatable and distasteful) on both colours (red and bronze).

| | Red common | Bronze common |
|---|---|---|
| Palatable | 29.85 ± 2.67 | 28.00 ± 1.83 |
| Distasteful | 35.74 ± 1.69 | 31.44 ± 1.35 |

**Table 4-12:** Frequency-independent selection (fixed frequency): repeated measures analysis for the effects of palatability, colour, site and order of presentation for red and bronze maggots on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Pal | 1 | 697.23 | 697.23 | 8.074 | <0.05 |
| Colour | 1 | 303.2 | 303.2 | 1.863 | n.s |
| Order | 1 | 195.87 | 195.87 | 1.203 | n.s |
| Site(FO) | 12 | 1952.793 | 162.733 | 1.885 | n.s |
| Pal*Colour | 1 | 48.22 | 48.22 | 0.558 | n.s |
| Pal*Order | 1 | 6.01 | 6.01 | 0.07 | n.s |
| Colour*Order | 1 | 197.81 | 197.81 | 1.216 | n.s |
| Pal*Colour*Order | 1 | 14.26 | 14.26 | 0.165 | n.s |
| Error | 12 | 1036.197 | 86.35 | | |

## 4-5- Discussion

In both experiments (1 and 2) the birds treated the maggots dipped in quinine solution as distasteful prey. They consumed the palatable maggots at an average rate that was higher than that for the distasteful maggots. Thus the tendency to avoid the distasteful prey was in agreement with similar experiments using live prey (Brower, 1960; Alcock, 1971; Sellén-Tullberg, 1985a; Hatle & Salazar, 2001) and, of course, artificial prey such as those used elsewhere in this thesis.

### 4-5-1- Frequency-dependent selection

Like many experiments that have investigated the effect of frequency-dependent selection using live prey (e.g. Pielowski, 1959; Ohguchi, 1978,1981; Wilson *et al.*, 1990), the results of these two experiments showed that selection on high densities of maggots was anti-apostatic. The most interesting result is that there was a significant interaction of palatability with frequency indicating that palatability does affect the direction and strength of frequency-dependent selection. This result is different from the results of previous experiments using artificial prey. Greenwood (1984) stated that predators could use cues from the prey individuals so that not only the conspicuousness of the rare form would help them to detect the prey but the prey's position can be fixed more easily, allowing them to pick the rare prey easily. This explanation suggests that if the prey were mobile, the strength of anti-apostatic selection will be increased by the movement of the prey. So if prey are moving and their density is high enough, there may be a confusion effect as well as selection against oddity and the confusion of so many moving prey may further increase the disadvantage of the rare morph (Greenwood, 1985; Landeau & Terbargh, 1986). Thus the finding that palatability affects selection on live prey but not artificial prey might be that the movement of the live prey affected the selection by the birds (Wilson *et al.,* 1990) and thus resulted in the birds being able to associate the colour of the rare form, if we assumed it was taken first, with distastefulness.

Lyytinen *et al.* (2001) conducted three experiments (in two experiments butterflies were glued on brown tray while in the third experiment butterflies were free to fly) to test whether European white butterflies *Anthocharis cardamines* and *Pieris napi* are aposematic. Only when the butterflies were free to fly (in the third experiment), did the wild pied flycatcher *Ficedula hypoleuca* often reject the butterflies suggesting that mobility might have led to the acceleration of the learning process of avoiding distasteful insects.

However, the effect of palatability was different in my two experiments. In experiment 1, selection was more anti-apostatic on distasteful prey, while in experiment 2, selection was more anti-apostatic on palatable prey. A possible reason could be that in experiment 1 when birds fed on the distasteful maggots, assuming that they tended to pick the rare form first (as it is the likely case in high densities of palatable prey), they quickly learned to associate distastefulness with the colour of the rare form. When the frequency was altered the birds tended to avoid this same colour, which was now common, and concentrated on the rare type. Thus anti-apostatic selection became stronger. This may explain why there was a significant interaction of palatability and frequency with order of presentation. On the other hand, birds which fed in the sites where the palatable prey were offered, behaved as would be expected when feeding on high density of palatable prey and took an excess of the rare form (Allen, 1972; Ohguchi, 1978, 1981; Allen & Anderson, 1984; Greenwood *et al.*, 1984a, 1984b; Allen *et al.,* 1998; Weale *et al.*, 2000). In experiment 2, birds may have picked the distasteful rare one first and after quickly learning that it was distasteful shifted their attack to the common form. However, upon learning that this colour was also distasteful they generalized. The generalization might have led the birds to increase their intake when the level of palatability was altered (birds were presented with palatable maggots). Thus the birds would have appeared to have a preference for the rare form of the palatable maggots, thus giving stronger anti-apostatic selection on the palatable maggots.

The effect of site on selection was only evident in experiment 1, where palatability was fixed. This could have been due to chance or, possibly, from the "confusion" resulting from the birds having to select from both levels of frequency in one site. Thus some birds, which had a preference for the rare form in the first trials for example, might have selected the new rare form regardless of the colour, while others continued to prefer the colour of the rare form which had become common in the last trials. Similar results were obtained by Horsley *et al.* (1979) from a similar design.

### 4-5-2- Frequency-independent selection

The preference for bronze over red in experiment 1 (fixed palatability) was similar to the preference for yellow over red in the experiments where the prey were artificial. However, it seemed that using live prey resulted in an interaction of both palatability and order of presentation on the consumption of bronze maggot. The birds consumed more bronze than red when maggots were distasteful than when they were palatable. This might indicate that birds were able to associate red with distastefulness faster than with bronze. However, in experiment 2 the birds failed to exhibit any preference for either colour, yet palatability had a significant effect reflecting an increased anti-apostatic selection on the palatable baits and, perhaps, that birds when they were offered distasteful maggots they took an excess of each colour when it was common.

### 4-5-3- Summary

The two experiments followed the designs used in chapter 1 but this time the prey were live maggots of *Calliphora vomitoria.* Both experiments demonstrated anti-apostatic selection and an effect of palatability on selection. However, the strength of anti-apostatic selection was different in the two experiments. In experiment 1, anti-apostatic selection was stronger on the distasteful maggots, while in experiment 2, anti-apostatic selection was stronger on the palatable maggots.

Only in experiment 1 was there an effect of frequency-independent selection: wild birds preferred bronze over red and tended to prefer it more when the maggots were distasteful.

# Chapter 5

## Polymorphism and heterosematism at high densities: *Umbonium vestiarium* and an experiment with pastry prey.

In this chapter, I change direction to consider a real prey species that lives at very high densities. This species, the marine snail *Umbonium vestiarium* is extremely polymorphic and thus seems to defy the prediction from the experiments presented so far that anti-apostatic selection should reduce the level of polymorphism.

## *5-1- Umbonium vestiarium*

### 5-1-1- Introduction

Populations of many animals exhibit considerable variation of coloration (Cain & Sheppard, 1950; Moment, 1962; Smith, 1971: Owen & Whiteley, 1986). Being different from conspecific neighbours would certainly make it hard for predators to form a search image (Allen, 1988b; Owen & Whiteley, 1988). However, the mechanism for maintaining such variation remains unknown. Many of the studies have shown that such polymorphism may be maintained by the selective predation by visual predators that hunt by sight such as birds, lizards and crabs (Sheppard, 1951; Cameron, 1969: Smith, 1975). Clarke (1962) was first to suggest that visual predators might maintain the colour polymorphism by frequency-dependent (apostatic) selection. Most of his argument concerned the polymorphic land snail *Cepaea nemoralis*. He argued that once the polymorphism has been established, they become more complex, with a rise in the number of forms. This could be the case for the polymorphism in *C. nemoralis* and other polymorphic species. Since then, many other studies, especially in snails (for example, the marine species *Littorina saxatilis* and *L. scabra*) have confirmed his argument (Heller, 1975; Cook 1983; Reid, 1987).

Owen (1963) studied the African land snail *Limicolaria martensiana* in the Kampala area in Uganda. He collected four different samples from four different sites and recognized four morphs: streaked, pallid 1, pallid 2 and pallid 3. The streaked form was always the commonest. He also found that the higher the density the higher the relative frequencies of the three pallid forms; in other words, the level of polymorphism increased with density. He proposed that polymorphism in populations of *L. martensiana* is maintained by apostatic selection and that the streaked form is at a selective advantage because of its cryptic coloration as long as the density is not above a certain critical level. Owen (1965) later obtained further data, this time from ten samples from the Buganda area and again drew the same conclusions. Greenwood (1969) reanalysed the data of Owen (1965) and concluded that the importance of apostatic selection is greater at intermediate densities than at high and low densities. He argued that at low and high densities, frequency-independent selection will be great enough to outweigh apostatic selection. Indeed, we now predict that selection at very high densities will be anti-apostatic (Allen, 1972; Horsley *et al.*, 1979; Allen *et al.*, 1998; Weale *et al.*, 2000; Church *et al.*, 2001) and this is another reason why polymorphism is expected to decrease at high densities. This chapter examines this prediction.

Polymorphism in Mollusca has been frequently studied in the field (Cain & Sheppard, 1950, 1954; Moment, 1962; Owen, 1965, 1969; Smith, 1971, 1975, 1976; Pettitt, 1973; Reid, 1987; Whiteley *et al.,* 1997; Johannesson & Ekendahl, 2002). Investigating frequency-dependent selection in natural populations is relatively difficult, partly because population densities tend to be low (Tinbergen *et al.*, 1967). However, dense assemblages are quite common in marine molluscs and other marine invertebrates (Reise, 1985). These aggregations are presumably formed by the molluscs fixing sediment with their mucus to help them building up a resistance to being washed away by the strong water current (Hüttel, 1986), or from the mode of feeding (Fedra, 1977), because they are particularly common in filter-feeding snails such as *Umbonium vestiarium* (L.), the species chosen for the study.

The snail *U. vestiarium* has a flat shell 7-12 mm in diameter (Fretter, 1975) and belongs to the family Trochidae, Archaegastropoda, Mollusca. Like *Cepaea nemoralis* populations, *U. vestiarium* populations contain several morphs whose frequencies can differ considerably between colonies populations. Endler (1978) argued that the frequency of morphs should be proportional to the frequency of the various background elements against which they are camouflaged. The reinforcement of any pattern would occur if there is frequency-dependent selection by the predator so that more of the common form is predated. However, *U. vestiarium*, like the bivalve *Donax variabilis* (Moment, 1962; Smith, 1971), lives in homogeneous fine sand and may lack the ability to be camouflaged. Allen (1988b) suggested that it is possible that selection on conspicuous prey living on homogeneous substrates would favour any individual that is different from the rest.

The morphs of *U. vestiarium* have been described as 'fine olive pattern', 'coarse olive pattern', 'wheel', 'white wash', 'white', 'pink spiral', 'pink spiral with accessory spiral', 'white spiral', 'pink all over', 'orange all over', 'orange spiral', 'candy stripe', 'pearl', 'pearl spiral', 'pink brown' and 'brown' (for more details see Grüneberg, 1980). Furthermore, their colour patterns are markedly different above and below the periphery of the shells ('heterosematism' Cain, 1984). *Umbonium* populations differ in size and colour from place to place (Grüneberg, 1980). There is so much variation within each form of the snails that it is often difficult to find any two individuals that are the same.

*U. vestiarium* has a wide geographical distribution, from Oman to Japan (Berry, 1987) and occurs on both rocky and sandy shores, though the latter shores are more preferred. It filter-feeds by burrowing into sand at the edge of the intertidal zone where waves break. In this surf-zone they often roll around like pebbles. It is an annual species, with recruitment occurring between March and June. The recruits grow for one year and die by the next recruitment (Berry, 1987).

Unlike the land snails, *Cepaea nemoralis* and *Limicolaria martensiana* (Owen, 1965; Greenwood, 1974), and the marine bivalves *Donax rugosus* and *Donacilla cornea* (Smith, 1971; Whiteley *et al.*, 1997), *U. vestiarium* tends to have very high population densities, sometimes exceeding 10,000 $m^{-2}$ (Berry, 1982). Berry (1982) found that the density of *Umbonium* varied from 3,299 $m^{-2}$ near the upshore edge of the sand flats to 22,635.5 $m^{-2}$ on the sand at 34 m inshore in Penang in Malaysia. Hüttel (1986) found even higher densities of 77,700 $m^{-2}$ in Phuket Island in Thailand.

The main aim of this work was to investigate whether there is a relationship between polymorphism and density in the *Umbonium vestiarium* population in Oman. We might predict that at extremely high densities selection will be anti-apostatic and thus such populations should tend to be less polymorphic.

## 5-1-2- Materials and Methods

The field work was carried out between 2nd December 2001 and 5th January 2002. *Umbonium vestiarium* occurs in various areas along the coast of Oman, and so seven beaches were chosen at random, although one beach, Masirah Island beach, was later excluded because of the occurrence of another similar species of *Umbonium*, *U. eloiseae* (Dance *et al.*, 1992) which was hard to distinguish from *U. vestiarium.* Two other beaches in the southern part of Oman were excluded because they were too far away.

### *5-1-2-1- The study sites*

#### 1- Al-Khuwair beach

Al-Khuwair beach is located in Al-Khuwair, a suburb of Muscat, the capital, of Oman. This brownish sandy beach is relatively commercial and it has an average of more than 1000 people visiting it per day. This probably limits the number of birds and other potential predators of *U. vestiarium* (for example, crabs) (Plate 5-1).

#### 2- Aswadi beach

This beach is 150 km to the North of Al-Khuwair beach on the Gulf of Oman. It is about 15 km long and used for both fishing activity and sunbathing. However, it is not as heavily visited as Al-Khuwair beach and so the number of birds is normally much higher than Al-Khuwair. The sand is brownish as in Al-khuwair beach, but has more small stones in some parts (Plate 5-2).

**Plate 5-1:** *Umbonium vestiarium* in Al-Khuwair beach. The beach floor is covered by hundreds of the snail shells of various colours and patterns.

**Plate 5-2:** *Umbonium vestiarium* in Ras Aswadi beach. The beach floor is covered not only by the snail shells but also by stones.

**Plate 5-3**: *Umbonium vestiarium* in Ras Al-Hid beach. Red and pink shells are very common.

**Plate 5-4:** *Umbonium vestiarium* in Barr Al-Hikman beach. The snail's crushed remains comprise most of the shells pile.

### 3- Ras Al-Hid (Sur) beach

This beach is located in the east of Oman on the Arabian Sea. It is about 20 km long and reaches another beach at Ras Al-Juniz, which green turtle, *Chelonia mydas*, use as a breeding site. The sand is reddish and fine. Like the Ras Aswadi beach, it is used for fishing and for picnics, which are relatively infrequent during the week days, and so human activities are normally limited to fishing in the early mornings and few hours in the afternoon. Hence, the number of sea birds is relatively higher than Ras Aswadi. It is also inhabited by crab species such as ghost crabs (*Ocypode* spp) and fiddler crabs (*Uca* spp) (Plate 5-3).

### 4- Barr Al-Hikman

This white sandy beach is on the Arabian Sea in the west of Oman. It is the longest beach in Oman and about 2 hundred kilometres in length. Human activities, mainly fishing, are limited. Thus the number of birds is extremely high, especially at the time the experiment took place when numerous migratory birds were seen. The number of crabs is also very high. Their tracks were obvious on the sand and there was much evidence of snail shells that had been crushed by the crabs (Plate 5-4).

### ***5-1-2-2- Method of sampling***

Equipment was limited. The basic method for collecting the snails was to draw on the sand a square of 60 cm × 60 cm about 5 m inside the surf. 20 samples were collected from a transect of 20 such squares, each 15 m apart and parallel to the shore. The total distance of the study area in each beach was thus about 300 m. All the work was carried out at midday at low tide and when there was limited human activity on the beach.

Since *Umboinum* snails live 5 mm to 15 mm below the sand (Berry & Othman, 1983) thus each sample was collected by removing the top 20 mm within each square using the hands. The contents of the pile were then put into a sieve (5 mm gauge) and then sieved in sea water, so that the only remaining content was shells and larger stones. The material was then placed in a labelled freezer bag. The *Umbonium* shells were later separated from the stones. Because the experiment was a preliminary study to emphasize the possibility that visual selection is occurring by looking at the frequency of each morph, or type as was classified in the experiment, it was less important that the majority of the samples collected were dead snails and empty shells.

Sorting was done on the intensity of the shell's colour, the patterns and the presence or absence of bands. Unaware of Grüneberg's (1980) classification of coloration in the genus *Umbonium*, these experimental samples were classified visually by a group of six people. We used human vision to classify *Umbonium* snails into groups as did Owen (1963, 1965) with *Limicolaria martensiana* and Hickson (1972) with *Theba pisana.* We assumed that predators such as sea birds would perceive the snails in a similar way.

We were able to identify over 47 different morphs based on both the upperside and underside colours and patterns; however we all agreed that they could be combined into five different visual types (Table 5-1, Plates 5-5 to 5-9). Many authors (Smith, 1971; Owen, 1963,1965; Hughes & Jones, 1985; Reid, 1987) have applied the same method and a recent example is that of Whiteley *et al.* (1997) who divided the massively polymorphic bivalve *Donacilla cornea,* which has over 25 forms, into 7 classes. A reason behind our decision to use five classes is that several morphs were extremely rare and only present in few sites within one beach or they were only found in one beach. Thus in order to include them in the method of analysis employed in this experiment we grouped them with other morphs whose colours and patterns they resembled.

**Table 5-1**: Colour and pattern of each type of the five types of *Umbonium vestiarium* snail involved in this experiment.

| Type | Description |
|---|---|
| A | Dark patterned shells (Olive, brown, brown-reddish or grey) see plate 5-5. |
| B | Light coloured and slightly patterned shells (red, pink, light-brown or light-grey) see plate 5-6. |
| C | Dark shells and some slightly banded (grey, black or blood-red) see plate 5-7. |
| D | White or pale shells see plate 5-8. |
| E | Spiral shells (brown, pink or patterned) see plate 5-9. |

**Plate 5-5:** Type A: dark patterned shells.

**Plate 5-6:** Type B: light coloured and slightly patterned shells.

**Plate 5-7:** Type C: dark shells.

**Plate 5-8:** Type D: white and pale shells.

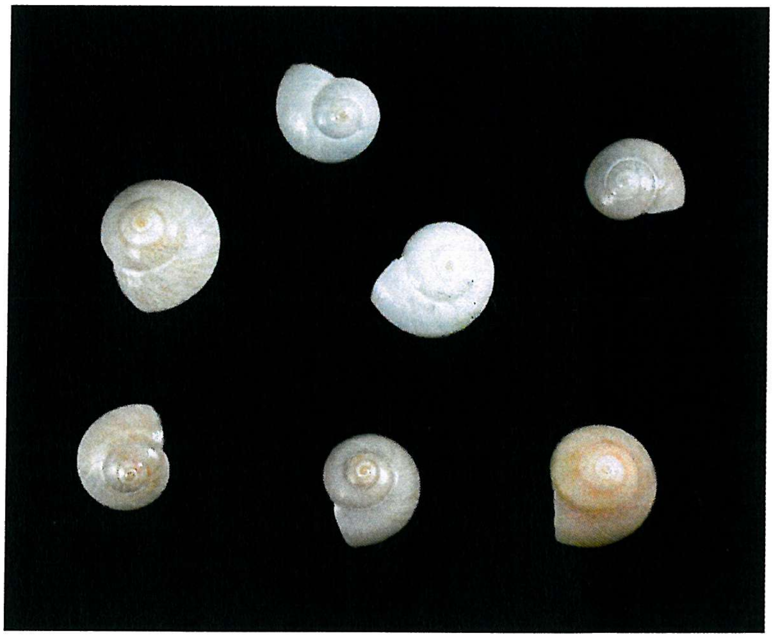


**Plate 5-9:** Type E: spiralled shells.

### *5-1-2-3- Method of Analysis*

The proportions of the five types among the sites within each beach and among the beaches were tested by Chi-square.

Diversity of the polymorphism was calculated for each sample and beach using Simpson's Reciprocal Index ($H=N(N-1)/(\Sigma\ n_i(n_i-1))$) where $n_i$ is the number of each type in the sample and N is the sample size. Diversity was then plotted against density to measure the correlation. In addition, GLM 'MINITAB', was used to investigate the effect of density on diversity within and among beaches. Density was arbitrarily divided into three levels: low, <85 $m^{-2}$; intermediate, between 85-155 $m^{-2}$, and high, <155 $m^{-2}$.

## 5-1-3- Results

A total of 8124 shells were collected from the four beaches. Over 47 different morphs were recorded from the 80 samples, with the highest number occurring in Al-Khuwair beach (38 forms). The lowest was Barr Al-Hikman (17 forms). Populations were typically comprised of three or four common forms in roughly equal frequencies, plus many rare. Of the four beaches studied, Barr Al-Hikman beach had on average the lowest densities of *Umbonium vestiarium*, while Al-Khuwair beach had on average the highest.

The percentage of the five types of *Umbonium vestiarium* in the 4 beaches are given in figs 5-1, 5-2, 5-3 and 5-4. Type A (dark patterned shells) had the highest percentage in all the sites of three beaches, Al-Khuwair, Ras Aswadi and Barr Al-Hikman. Ras Al-Hid beach had a high percentage of type B, which comprises light coloured patterned shells, red and pink shells, but this was among the rarest types in Al-Khuwair and Ras Aswadi. The other three types had lower percentages at all the four beaches. There seemed to be a negative correlation between type A and type E (banded shells) and a slight correlation between type A and type B, in which an increase of one tended to give a rise for the other one especially in Al-Khuwair, Ras Aswadi and Sur.

Table 5-2 gives the raw data for the number of the five types at the four beaches and summaries the chi-square analysis. Within beaches, there was significant heterogeneity among sites at Al-Khuwair, Ras Al-Hid and Barr Al-Hikman beaches.

Using the total numbers of the five forms at each beach, there was a highly significant difference between the proportions of the five forms at the four beaches ($\chi^2_{(12)} = 1421.4$, $P<0.01$).

Fig 5-5 shows the relationship between diversity and density for the four beaches. On average Ras Al-Hid beach had the lowest diversity although Barr Al-Hikman had on average the lowest densities. ANOVA confirmed that there is highly significant differences between beaches ($F_{3,20} = 34.81$ $P<0.001$). There was no effect of density on diversity ($F_{2,20} = 0.631$ $P>0.05$ ) (Table 5-3).

**Figure 5-1**: The % of each type of the five types of *Umbonium vestiarium* in the 20 sites within Al-Khuwair beach.

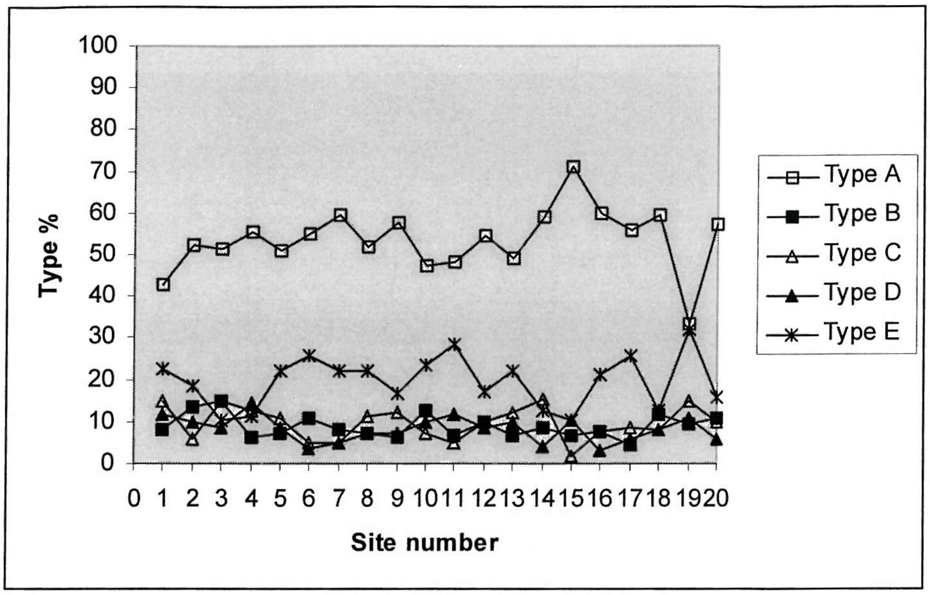


**Figure 5-2**: The % of each type of the five types of *Umbonium vestiarium* in the 20 sites within Barr Al-Hikman beach.

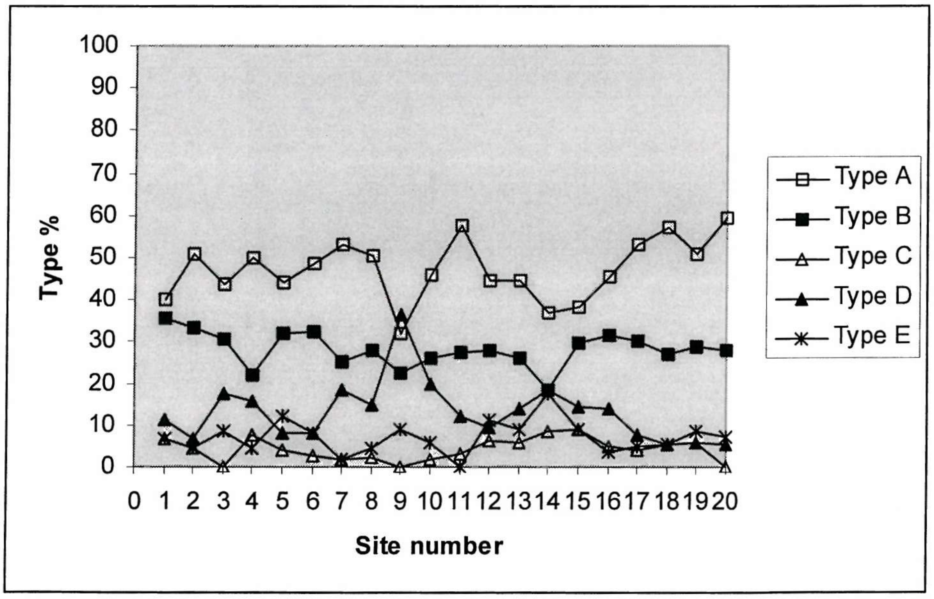


**Figure 5-3**: The % of each type of the five types of *Umbonium vestiarium* in the 20 sites within Ras Aswadi beach.

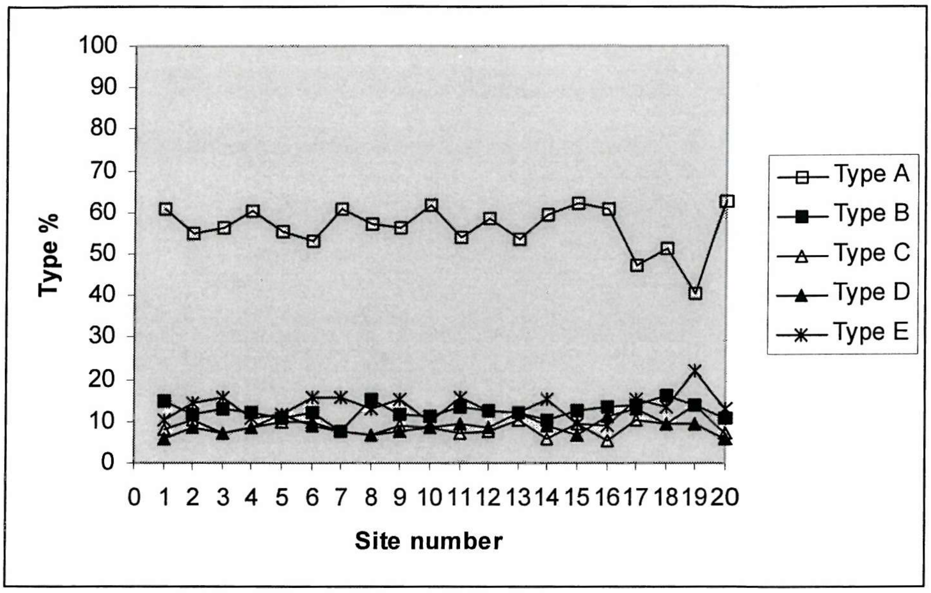


**Figure 5-4**: The % of each type of the five types of *Umbonium vestiarium* in the 20 sites within Ras Al-Hid beach.

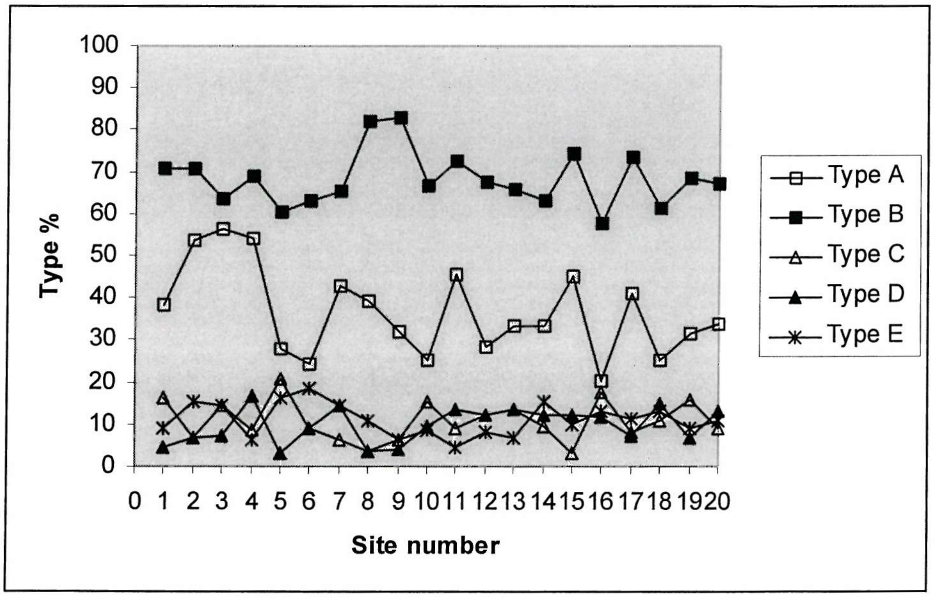


**Figure 5-5**: The diversity of each sample of *Umbonium vestiarium* plotted against density $(m^{-2})$ within all the 4 beaches. Trendlines illustrate the correlation between density and diversity for each beach.

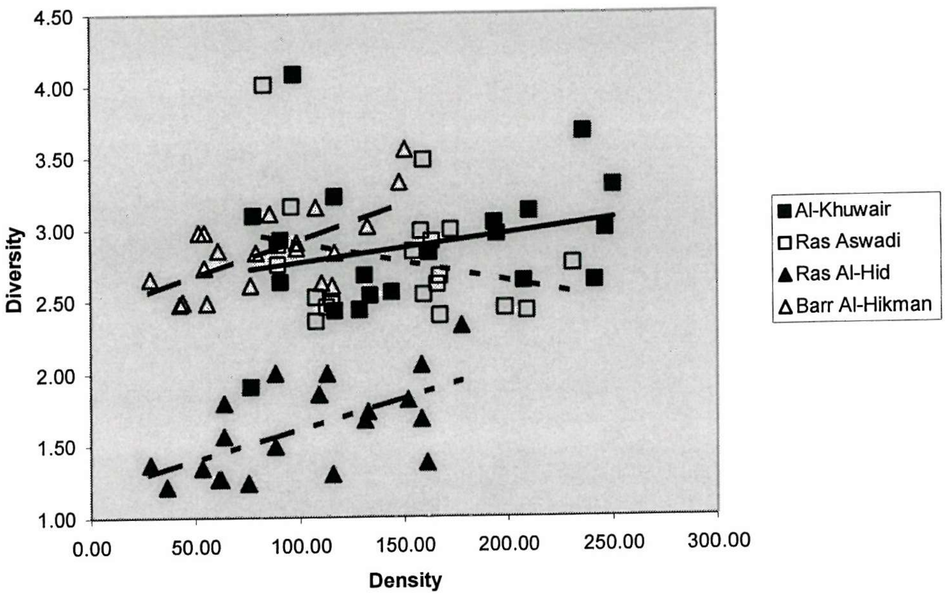


**Table 5-2:** Total numbers of types A-E at the four beaches, $\chi^2$ and P values in two right hand columns are for heterogeneity among sites within each beach.

| | Phenotypes | | | | | | |
|---|---|---|---|---|---|---|---|
| Beach | Type A | Type B | Type C | Type D | Type E | $\chi^2_{(76)}$ | P |
| Al-Khuwair | 1284 | 227 | 242 | 201 | 471 | 134.57 | <0.05 |
| Ras Aswadi | 1272 | 276 | 193 | 200 | 301 | 44.79 | n.s |
| Barr Al-Hikman | 643 | 376 | 64 | 172 | 100 | 134.09 | <0.05 |
| Ras Al-Hid | 534 | 1046 | 177 | 160 | 180 | 258.94 | <0.01 |
| **Total** | **3733** | **1925** | **676** | **733** | **1052** | | |
| $\chi^2_{(12)}$ among beaches = 1421.4, P<0.001 | | | | | | | |

**Table 5-3**: GLM analysis for the effect of beach, site and density on diversity

| Source | df | SS | MS | F | Significance |
|---|---|---|---|---|---|
| Beach | 3 | 11.474 | 3.825 | 35.724 | <0.001 |
| Site | 19 | 3.525 | 0.186 | 1.733 | n.s |
| Density | 2 | 0.122 | 0.061 | 0.572 | n.s |
| Beach*Density | 4 | 0.627 | 0.157 | 1.464 | n.s |
| Site*Density | 28 | 3.128 | 0.112 | 1.043 | n.s |
| Error | 22 | 2.355 | 0.107 | | |

## 5-1-4- Discussion

The most likely explanation for the high relative frequencies of types A and B was that they were cryptic against the background (at least to the human eye). Studies on *Cepaea* have demonstrated that snails with a yellow shell ground are the most frequent in populations living on grass (Sheppard, 1951; Cain & Sheppard, 1954). Many other studies have reported similar correlations between the frequencies of morphs and the visual properties of the backgrounds (Heller, 1975; Hughes & Jones, 1985; Reid, 1987). For example, Reid (1987) reported that yellow shells of the mangrove snail *Littoraria filosa* were more frequent on the mangrove tree *Avicennnia* with abundant yellowish green foliage, while brown shells were abundant on relatively bare trees.

Ras Al-Hid (Sur) beach has a reddish sand for which the frequency of pink, red and light brown (type B) was higher than type A, which was the commonest type in the other three beaches. Moreover, the whitish sand of Barr Al-Hikman correlates with a relatively high percentage of white and pale shells (type D) which is usually the rarest type at the other beaches.

These data contradict Grüneberg (1980). He collected 11 *Umbonium* samples, and classified them into four geographical categories; India and Ceylon, Ganagavaram, Hong Kong and Penang. Although some morphs were found in all the four areas (e.g. 'candy stripe', 'white spiral' and 'bronze/brown stripe'), there were some morphs that were restricted to one area and not the others. Examples are red shells in Hong Kong, pink shells in India and bronze/olive shells in Penang. He claimed that polymorphism in

*Umbonium* cannot possibly result from apostatic selection because the snail has no visual predators as it is always buried under the sand. Indeed he discounted selection of any sort as being responsible for maintaining the polymorphism. Instead he suggested the polymorphism is the result of gradual or abrupt changes during growth and therefore, should be considered a 'pseudo-polymorphism'. It is true that shell colour does seem to change if growth is interrupted due to injuries or other diseases (personal observations). However, Grüneberg failed to study the nature of the background of the sites where the samples were collected.

In common with the polymorphic intertidal bivalve *Donax rugosus* (Smith, 1971), *U. vestiarium* is buried for most of the time beneath the surface of the sand. However, the snails are exposed to visual predators when the surf breaks over them and moves them, like pebbles, up and down the beach. In this study, Barr Al-Hikman, which is inhabited by large numbers of birds and crabs which are known to feed on snails (Croze, 1970; Pettitt, 1973; Reid, 1984; Yamada *et al.*, 1998; Copello & Favero, 2001), interestingly had on average the lowest densities of *Umbonium*. Al-Khuwair, which had the least number of birds, had the highest densities and the number of forms. This could mean that there is an effect of predation on both the size of the snail population and the number of morphs within each beach. Clearly, more work is needed.

This study showed that the frequency of each of the five types varied from site to site within all the beaches apart from Ras Aswadi. Similar results have been obtained by Hickson (1972) who collected 16 samples of the land snail *Theba pisana* from the False Bay in South Africa. He found significant variation between the samples (sites) with respect to white, pale and dark shells. Since there was no obvious visual predation nor there was any selective effect of the environment thus he suggested that random effects were responsible for such variation in shell colour frequencies. However, Lewis (1977), who also found that the frequency of each of the four types (discontinuous opaque one-banded, discontinuous opaque more-than-one-banded, continuous opaque one-banded and continuous opaque more-than-one-banded) of the land snail *Cochlicella acuta* varied between samples within one colony, suggested that visual predation, especially by rooks *Corvus frugilegus* was partly responsible for such variation. Cook (1986) also found significant variation in frequency of the mangrove snail *Littoraria pallescens* which he attributed to visual predation. In this study we did not observe any predation upon the

*Umbonium* snail although we assume that the crushed remains of this snail in Barr Al-Hikman were the result of crab predation. Thus it is possible that visual predation is partly responsible for the variation of frequencies of the five types among the sites.

Whiteley *et al.* (1997) collected samples of *Donacilla cornea* from 4 beaches (Korinos and Kitros, Greece; Argelès, France; Aldán, Spain) in the Mediterranean sea. They found that morph frequencies and population diversity differed significantly from beach to beach. They suggested that this might be attributed to the interaction of frequency-dependent and frequency-independent selection under the mechanisms of crypsis (similar range of colour and pattern variation between shells and substrate) and apostatic selection (there were one or two common phenotypes and a number of rare ones). Since the results of this study also showed significant variation among beaches thus this also might indicate the effect of frequency-dependent selection

Al-Khuwair and Ras Aswadi populations are so close to each other that there must be high a likelihood of exchanging genes between the two. Indeed there is a striking similarity between the two in the number of forms, percentage of each type and population density. The difference in their type frequencies could perhaps be attributed to the presence of the small stones, which are only found in Ras Aswadi, that could act as a matching background for some of the forms.

In this study there was significant variation in diversity among the beaches. However, there was no evidence of the effect of density on diversity as suggested by Owen (1965, 1969) working on the African land snail *Limicolaria martensiana* and Smith (1971) working on the intertidal bivalve *Donax rugosus*. These results are in agreement with the results of the work by Whiteley *et al.* (1997) on *Donacilla cornea*. Using the Shannon-Wiener diversity index they found that the highly dense Korinos population of 910.4 $m^{-2}$ had an equivalent diversity to Kitros and Aldan populations with a density of 166.6 $m^{-2}$ and 88.6 $m^{-2}$ respectively. Thus they also found no evidence for an effect of density on diversity. However, there is a case for sampling many more populations of *U. vestiarium* because of the suggestion of a positive relationship of diversity and density (e.g. at Ras Al-Hid, fig 5-5)

Greenwood (1969) predicted that apostatic selection, which may be responsible for maintaining polymorphism within a population of prey, will be greatest at intermediate densities and least at low and extremely high density. The results of this work on *Umbonium* suggest that the highest diversity value was at an intermediate density while the lowest diversity value was at a low density. This agrees with Greenwood's predication. However, the population at Barr Al-Hikman beach, which on average had the lowest density, had a diversity similar to that of Al-Khuwair beach and Ras Aswadi beach, both of which, on average, had an intermediate density.

Spawning and mode of larval development are important factors influencing the genetic structure of marine adult populations. The colour variations in the snails are the result of mutations in several genes responsible for the pigmentation for both the shell and the body. Many genes influence the expression of single genes so that the intensity of expression can vary even for normal treats. As far as I know there is not much known about *Umbonium* snail genetics, and certainly the genes that control the colour. Larval development time of trochacean species can be highly dependent on temperature and wave action (Grosberg & Levitan, 1992). Thus it seems likely that the larvae have the potential for substantial dispersal in the field. Marine species with long-lived planktotrophic larvae generally disperse more broadly and show much less population differentiation over large special scales than species that lack a planktonic larval stage (Moran, 1997). However, the planktonic stage of *Umbonium* lasts only a few days before settlement as crawling juveniles (Berry, 1986). Spawning takes place between April and August during which the tide is considerably low (Berry, 1987). Thus it seems likely that the tidal regime might directly favour the settlement, survival and growth of early young. However, other factors could be of a great importance in causing the massive varition of this snail, for example genetic drift, gene flow, wave action, salinty of water, nature of predation, if any, and of course the temprature. Thus clearly there is a need for more information about all these factors to be able to draw a meaningful conclusion.

One characteristic of *U. vestiarium* that was not investigated is 'heterosematism' (Cain, 1984). Each snail tends to be very different visually when seen from below compared with above. For example, some of the dark shells show white, grey, pink or a mixture of two colours from underside.

*U. vestiarium* is very prone to being turned over in the surf and is a likely candidate for the evolution of heterosematism (Cain, 1984). If a predator fixes its attention to a particular individual and this individual is turned over by the surf it will have a chance of surviving if its ventral coloration is then not recognized by the predator. Heterosematism may thus increase the amount of diversity within the population and increase the strength of frequency-dependent selection. The next section describes an attempt to test this using artificial prey.

## ***5-2- Heterosematism***

### 5-2-1- Introduction

Many invertebrates have an underside that is coloured differently from the upperside and this has been termed 'heterosematism' by Cain (1984). Examples include species of snails and moths (Cain, 1984; Owen & Whiteley, 1989). Furthermore, the coloration of the underside sometimes differs significantly from individual to individual within populations of the same species (Sargent, 1978). Owen & Whiteley (1989) recognises that polymorphisms of the upper and lower surfaces can lead to massive variation in some species of moths, for example, *Noctua fimbriata*, *Anticarsia gemmatilis* and *Salamis cacta*. It has been suggested that the coloration of the underside acts as 'flash coloration', which is a defensive mechanism used by the prey to startle their predators and thus increase the chances of escaping the attack (Edmunds, 1974). The more variable the underside colour, the more effective are the startle devices (Sargent, 1973, 1978). Studying the morph frequencies of palatable *Catocala* moths in North America, Sargent & Owen (1975) speculated that its underside wings were subjected to frequency-dependent selection and the advantage to a particular morph is reduced when its frequency exceeds 25%.

The variable underside coloration of some snails, such as *Umbonium vestiarium* in the previous study, is presumably not an example of flash coloration of the sort found in some lepidoptera. This is because one would expect that such coloration should be made visible by the snail quickly, with it deliberately turning over to increase the likelihood of scaring away the attacking predator. As far as I am aware, such behaviour never occurs in gastropods. Although the underside coloration of snails varies considerably from cryptic to conspicuous (for example in *Cepaea*, Cain, 1984), it is probably never as massively variable as, for instance, in *Catocala*.

However, the ventral coloration of snail shells might still work to mislead predators. Cain (1984) was a pioneer in studying heterosematism in snails. He noticed that in both equi-dimensional and depressed-shelled species the aperture region of the shell and, in particular, the umbilical region of tall-shelled species are often distinctively coloured or patterned. These regions are normally hidden when the snail is fixed to the substrate. Cain postulated that a predator attacking a snail would notice the dorsal coloration of the shell. If the snail fell (e.g. from the bill of the bird) then it would be more likely to escape subsequent detection if it fell upside down and revealed its differently coloured ventral surface. He used 18 live specimens of different species of land snail and allowed them to drop from two different heights (10 cm and 20 cm) and land on a flat cloth spread on a bench-top. The holding positions of the equi-dimensional and depressed shells, when positioned to drop, were 'apex up', 'apex down' and 'broad side down' whereas the holding positions of the tall shells were 'apex up' and 'apex down' only. The results showed that there is 50:50 chance that the individual snail would rest with either side facing upwards. This ratio would be sufficient for the evolution of heterosematism (Cain, 1984).

Thus it is possible that the presence of two different colours on each side of the *Umbonium* snail is deliberately misleading to predators. A predator which has adopted a searching image to an individual's common upperside colour would be momentarily confused if that particular individual snail was turned over by the water surf. Thus the main aim of this experiment was to investigate the effect of such heterosematism on frequency-dependent selection at high density of artificial pastry prey. We might predict that heterosematism will increase the effective diversity of the prey and thus selection will be apostatic.

## 5-2-2- Materials and Methods

The baits were made from plain flour and lard in a 3:1 ratio (see chapter 2). Edible colouring dye (Pointing Ltd) of 20 ml strawberry red (K6020) or 16 ml egg yellow (K6013) were added to give the required colours. However, this time the dough (750 g of flour and 250 g of lard) was rolled out on the work-top to a thickness of 6 mm thick using a wooden roller. The dough was then cut into 10 mm × 6 mm × 6 mm square shapes.

The upper and lower sides of the pastry were coloured differently by spraying the top of the dough, before it was cut, with either red if the dough was yellow or yellow if it was red. The spray was made by mixing 30 ml of water with 20 ml of the required colour in a spray can. The spraying procedure started with the application of a small quantity of the spray on the top of the dough and then left to dry for 2 hours before cutting the dough into squares. Red dough was first coated by white, made of 20 ml pearl white with 30 ml of water, and left for two hours before spraying with yellow. This was done to guarantee a suitable yellow underside colour. Hue and value/chroma estimates (Munsell Book of Color, 1967) for one-coloured baits and the upperside were: red 5R 5/12 and yellow 10YR 8/12, while the estimates for the undersides were: red 7.5R 5/16 and yellow 2.5YR 5/16. This difference in the chroma and hues was not critical because the birds at any one site were not exposed to either both types of red or either both types of yellow.

The baits were presented in 9:1 ratios so that a 'one-coloured common' population was 180 one-coloured and 20 two-coloured and a 'one-coloured rare' population was 20 one-coloured and 180 two-coloured.

### 5-2-2-1- Predators

The birds were free ranging wild birds and included blackbirds *Turdus merula*, house sparrows *Passer domesticus*, robins *Erithacus rubecula*, wood pigeons *Columba palumbus*, starlings *Sturnus vulgaris*, great tits *Parus major*, blue tits *P. caeruleus* and magpies *Pica pica*.

### 5-2-2-2- Design

The experiment was carried out in the Southampton, Winchester and Eastleigh areas between 20th May and 12th September 2002. Similar in design to the experiments described in chapters 2, 3 and 4, the experiment had a split-plot design with two levels of prey frequency (one-coloured common and one-coloured rare), two orientations (underside facing upwards and underside facing downwards), two levels of order of presentation at each site (upwards first followed by downwards and downwards first followed by upwards) and four trials per level per site (Table 5-4). Thus in total there were four treatments, representing the four possible combinations of frequency and order of presentation: (a) 90% one-coloured with facing upwards first (b) 90% one-coloured with facing upwards second (c) 10% one-coloured with facing upwards first (d) 10% one-coloured with facing upwards second. Each level of orientation was presented in four successive trials (for either red or yellow) per site, giving a total of 192 trials for the whole experiment. It must be mentioned that one-coloured baits can be either red or yellow and two-coloured baits can be either red with the underside coloured yellow or yellow with the underside coloured red (Figure 5-6).

**Figure 5-6:** The orientation of one-coloured and two-coloured baits.

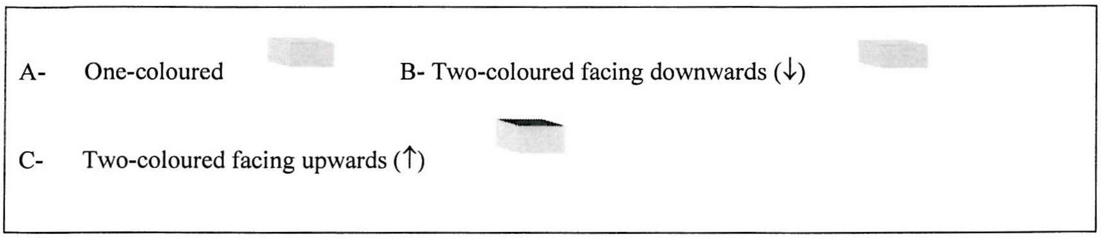


**Table 5-4:** Experimental design. (→) refers to followed-by.

- 4 treatments
  - -Treatment 1: 10% of two-coloured-upwards → 10% of two-coloured-downwards
  - -Treatment 2: 10% of two-coloured-downwards → 10% of two-coloured-upwards
  - -Treatment 3: 90% of two-coloured-upwards → 90% of two-coloured-downwards
  - -Treatment 4: 90% of two-coloured-downwards → 90% two-coloured-upwards
- 3 sites / treatment = 12 sites
- 2 combinations of frequency and orientation/ treatment
- 4 trials/ combination of frequency and orientation/site = 96 trials
- 96 trials/ colour = 192 trials

### *5-2-2-3- Analysis*

Overall selection against one-coloured was measured by Manly's (1973, 1974) β coefficient using the same formula used in previous experiments:

$$\beta = \log(C_1/c_1) / [\log(C_1/c_1) + \log(C_2/c_2)]$$

Where $C_1$ = number of one-coloured presented
$c_1$ = number of one-coloured left
$C_2$ = number of two-coloured presented
$c_2$ = number of two-coloured left

Repeated measures analysis was then applied to arcsine transformed β and site was nested within frequency, colour and order of presentation.

### 5-2-3- Results

The birds exhibited highly statistically significant frequency-dependent selection (frequency main effect: $F_{1,16} = 17.543$, $P<0.001$). Overall selection was apostatic with the average β value (51.68 ± 2.08) high when one-coloured baits were common and low (34.34 ± 1.47) when one-coloured baits were rare (Table 5-5). However, there was a highly significant interaction of frequency with orientation (main effect: $F_{1,16} = 10.164$, $P<0.001$). Selection when the underside was facing downwards was more apostatic at both levels of frequency (Table 5-5). The results of the statistical analysis are given in Table 5-9.

**Table 5-5:** Means (±S.D.) of transformed β (selection against one-coloured) for both orientations of two-coloured (underside-facing-upwards and underside-facing-downwards) at both frequencies (one-coloured common and one-coloured rare).

| | Two-coloured upwards | Two-coloured downwards |
|---|---|---|
| One-coloured common | 45.57 ± 4.48 | 57.79 ± 2.38 |
| One-coloured rare | 38.84 ± 4.29 | 29.83 ± 2.65 |

The interaction of frequency with order was very highly significant ($F_{1,16} = 25.11$, $P<0.001$), with selection being apostatic only when upwards presented second (Table 5-6).

Table 5-7 shows that when the upwards orientation was presented first, selection was anti-apostatic. However, in all other cases (i.e. when upwards orientation was presented second, downwards orientation first and downwards orientation second), selection was apostatic. Thus there is an extremely highly significant interaction of frequency, position and order of presentation ($F_{1,16} = 46.991$, $P<0.0001$).

**Table 5-6:** Means (±S.D.) of transformed β (selection against one-coloured) for both frequencies (one-coloured common and one-coloured rare) and when upwards orientation was first and when was second.

| | Two-coloured-upwards presented 1st | Two-coloured-upwards presented 2nd |
|---|---|---|
| One-coloured common | 41.08 ± 3.48 | 62.28 ± 3.23 |
| One-coloured rare | 44.48 ± 3.26 | 24.19 ± 3.37 |

**Table 5-7:** Means (±S.D.) of transformed β (selection against one-coloured) for both frequencies (one-coloured common and one-coloured rare) when either level of orientations of two-coloured (underside-facing-upwards and underside-facing-downwards) was first and when was second.

| | Two-coloured upwards | | Two-coloured downwards | |
|---|---|---|---|---|
| | Presented 1st | Presented 2nd | Presented 1st | Presented 2nd |
| One-coloured common | 23.35 ± 3.37 | 67.78 ± 5.27 | 56.79 ± 3.51 | 58.80 ± 3.29 |
| One-coloured rare | 60.20 ± 1.99 | 17.48 ± 5.62 | 30.91 ± 3.28 | 28.76 ± 4.23 |

The results showed that when baits were yellow, selection against one-coloured was higher when both upwards and downwards orientations were second. There seems to be a preference for red when upwards orientation was presented first (Table 5-8). Interaction of colour, orientation and order was statistically significant (main effect: $F_{1,16} = 8.728$, $P<0.01$). Apart from frequency, there was no significant effect of each factor alone including site.

**Table 5-8:** Means (±S.D.) of transformed β for both orientations of two-coloured (underside-facing-upwards and underside-facing-downwards) and both colours (red and yellow) at two levels of presentation (upwards first and upwards second).

| | Two-coloured upwards | | Two-coloured downwards | |
|---|---|---|---|---|
| | Presented 1st | Presented 2nd | Presented 1st | Presented 2nd |
| Yellow | 35.98 ± 4.37 | 50.48 ± 6.70 | 45.33 ± 3.92 | 51.30 ± 4.92 |
| Red | 47.57 ± 4.78 | 34.77 ± 8.01 | 42.35 ± 4.69 | 36.98 ± 4.39 |

**Table 5-9:** Frequency-dependent selection: repeated measures analysis for the effects of frequency, orientation, colour and order of presentation for heterosematic prey on transformed β values.

| Source | df | SS | MS | F | P |
|---|---|---|---|---|---|
| Freq | 1 | 14437.249 | 14437.249 | 17.543 | <0.001 |
| Orientation | 1 | 124.791 | 124.791 | 0.234 | n.s |
| Colour | 1 | 1468.020 | 1468.020 | 1.784 | n.s |
| Order | 1 | 10.007 | 10.007 | 0.012 | n.s |
| Site(Freq*Col*Order) | 16 | 13167.298 | 822.956 | 1.574 | n.s |
| Freq*Orientation | 1 | 5409.626 | 5409.626 | 10.164 | <0.01 |
| Freq*Colour | 1 | 1295.040 | 1295.040 | 1.087 | n.s |
| Orientation*Colour | 1 | 578.288 | 578.288 | 0.974 | n.s |
| Freq*Orient*Colour | 1 | 518.261 | 518.261 | 1.546 | n.s |
| Freq*Order | 1 | 20662.516 | 20662.516 | 25.108 | <0.001 |
| Orientation*Order | 1 | 7.361 | 7.361 | 0.014 | n.s |
| Freq*Orient*Order | 1 | 25009.245 | 25009.245 | 46.991 | <0.0001 |
| Colour*Order | 1 | 696.522 | 696.522 | 0.846 | n.s |
| Freq*Colour*Order | 1 | 831.287 | 831.287 | 1.01 | n.s |
| Orient*Colour*Order | 1 | 4644.938 | 4644.938 | 8.728 | <0.01 |
| Freq*Orient*Col*Order | 1 | 222.126 | 222.126 | 0.417 | n.s |
| Error | 16 | 8515.372 | 532.210 |  |  |

## 5-2-4- Discussion

This experiment demonstrated that selection on prey with a different underside coloration is frequency-dependent. Many experiments, including those described elsewhere in this thesis, have shown that selection on palatable dimorphic prey at high densities is usually anti-apostatic (Allen, 1972; Horsley *et al.* 1979; Allen *et al.*, 1998; Weale *et al.* 2000). However, the results for this particular experiment suggest that selection was apostatic.

The significant interaction of frequency with order of presentation suggests that when the underside of the prey was facing downwards and so both prey types looked to have the same colour, the predators established a preference towards that colour (red or yellow). Thus in the following trials they ignored what would appear to be the novel underside colour when it was facing upwards. Therefore, selection seemed to be extremely apostatic when facing upwards was second. The birds were effectively 'trained' to one colour. Several experiments have shown that birds acquire preferences for the familiar forms (Allen & Clarke, 1968; Allen, 1974, 1984; Harvey *et al.*, 1974; Raymond, 1984; Allen *et al.*, 1988). In these experiments after familiarizing the birds with one form, they were later given an equal proportion (50:50 ratio) of two colours including the familiar one.

The results demonstrated a significant interaction between frequency, orientation and order of presentation. The strength and direction of frequency-dependent selection varied according to the combination of these three factors. When one-coloured was common and the underside of the two-coloured was facing downwards the birds exerted apostatic selection. This is because both baits appeared to have the same colour (either red or yellow) and the rare form was few in number (10 %) and intermingled with the common form. Thus the birds fed randomly but, perhaps through chance, took more of the common form. When the orientation was altered (the underside was facing upwards) the birds exerted stronger apostatic selection, tending to ignore the rare form (the novel underside colour after the birds were accustomed to the familiar upperside colour). This avoidance may well indicate that if the underside is differently coloured ('heterosematic'), the snail might escape untouched if it was dropped from the bird's bill, had the bird formed a preference 'searching image' to the familiar upperside colour, and fell down exposing the underside colour (Cain, 1984).

The change in direction of frequency-dependent selection was noticed in the trials when the underside colour facing upwards was presented first, whether one-coloured was common or rare. The birds fed, as expected, anti-apostatically (Allen, 1972; Horsley *et al.* 1979; Allen *et al.*, 1998; Weale *et al.* 2000) but then when the orientation was altered (underside facing downwards) in the following trials, they exerted apostatic selection. Thus overall selection became neutral.

The significant interaction of colour, orientation and order of presentation suggests that birds colour preferences was different in both levels of orientation and at different orders of presentation. Birds preferred red only when the underside facing upwards was presented first but not when presented second (novel). This may suggest that, as mentioned in previous experiments which used red and yellow, that red is more powerful than yellow in averting the birds from attacking.

The results of this experiment suggest that being heterosematic the rare morphs of the *Umbonium* snail will be at a selective advantage have their predators established searching image for the upperside.

### *Summary*

Selection on the high density of heterosematic artificial prey was apostatic. Thus heterosematism may increase diversity because predators after being accustomed to the familiar upperside colour they tended to avoid the novel underside colour.

# Chapter 6

## Main Discussion

### *6-1- Introduction*

In this final chapter I bring together the main points made in the previous chapters. In three chapters, 2, 3, and 4, seven experiments were designed to investigate the effect of palatability on selection. In these experiments I always used a high density for two reasons. First, to investigate selection when the prey are distasteful, as it is expected that at a high density of palatable prey selection would be anti-apostatic (Allen, 1972; Horsley *et al.*, 1979; Willis *et al.*, 1980; Allen *et al.*, 1998; Weale *et al.*, 2000). Second, it is known that aposematic species, which use their distinct coloration as a warning signal for their distastefulness, often live at high densities (Edmunds, 1974).

The experiments reported in chapter 2 were designed to show the relative risks to the distasteful rare prey in the different designs imposed in experiments 1 and 2 and the different colours imposed in experiment 3. Results from experiments 2 and 3 showed that selection was anti-apostatic whether the prey were palatable or distasteful but there was no significant effect of palatability.

In chapter 3, the experiment with captive birds had the same design as experiment 1 in the previous chapter, but still demonstrated anti-apostatic selection. In the other experiment in chapter 3, a video camera was used to record as many individual predator/prey encounters as was feasible and consequently it was important to ensure the repeated attendance of target species and individual birds. Selection by robins, house sparrows, individual robins and individual blackbirds also demonstrated anti-apostatic selection. An effect of palatability was only noted for selection by blackbird individuals.

The two experiments in chapter 4 had the same designs as experiments 1 and 2 in chapter 2 but live prey were used instead. Wild birds also fed anti-apostatically on the dyed maggots *Calliphora vomitoria.* Palatability affected the strength of anti-apostatic selection but was different in each experiment.

Chapter 5 departed from the effect of palatability on selection but still explored frequency-dependent selection at high density. In one experiment samples of the intertidal polymorphic snail, *Umbonium vestiarium* were collected from 4 different beaches in Oman to determine if anti-apostatic selection was really acting. The results showed that there was no difference in diversity index between the three densities. In another experiment with a high density of artificial pastry prey, heterosematism seemed to promote apostatic selection.

## 6-2- General trends in predation

The level of palatability influenced the degree of predation on artificial and live prey and this could be seen in the difference in the rate of predation between the two forms of the palatability. In most of the experiments the rate of predation (i.e. number of baits taken/hour) on the palatable form was higher than the rate of predation in the unpalatable form. However, in the two experiments which dealt with captive birds and the known bird species, palatability seemed to have no effect on the predation rate. This presumably could have been to birds feeding on the baits for the purpose of sampling or could have been due to hunger if one takes into account that the captive birds had a regular access to food all day long prior to the commencing of the trials. Hunger and the presence of larger birds might have been the reason for the target species failing to show any difference in the rate of predation between the two levels of palatability although the difference in the number of baits taken per visit between the palatable and distasteful baits was significant for two bird species; robins and blackbirds.

Heterogeneity of selection among sites is usually expected when one is dealing with assemblage of wild birds (Horsley at el., 1979). This is partly because each bird species tend to have their own technique of perceiving and approaching a food item (Krebs, 1978; Greenwood, 1984; Stephens & Krebs, 1986), even at an individual level, as for example, in individual blackbirds (Allen & Anderson, 1984). Furthermore, Church et al. (1997) investigated the effect of dispersion on frequency-dependent selection in

Southampton (England) and Aljarafe (Spain) and found stronger anti-apostatic selection in Southampton. They attributed this to two probable reasons, but both related to the nature of the predators at the two sites. However, in my experiments only twice was heterogeneity among sites observed when testing for frequency-dependent selection; in the green and brown experiment in chapter 2 and in the fixed palatability experiment with live prey in chapter 4. This may suggest that wild birds were confused in their selection behaviour upon finding green and brown, colours which are normally associated with edible prey in their surrounding, distasteful, and thus their selection differed from site to site. Confusion might have also been the reason for the significant heterogeneity in the fixed palatability/live prey experiment (chapter 4) in which the effect of palatability was significant. Wild birds were confused to find the frequency of the maggots was altered. Regular predators, on their arrival at the site, may have expected to find the frequency of the rare morph as that they had last experienced. Thus at some sites birds, which were able to associate the colour of the previous rare form with distastefulness, started feeding on the new rare form which had the other colour. At other sites birds fed on the new common form, perhaps, because they had preference for the colour irrespective if it was the colour of the rare or common forms.

The density of *Umbonium vestiarium* seemed to be correlated with the number of birds and crabs present in each beach. The highest densities occurred at Al-Khuwair beach which had the least number of birds and crabs while the lowest ones occurred at Barr Al-Hikman which had the highest number of birds and crabs. In both those two beaches the diversity of the snail seemed to be similar, while the lowest diversity occurred at Ras Al-Hid beach where the density of snails was moderate. This seems to be different from the assumption of Greenwood (1969) who has speculated that diversity tends to be highest at moderate densities and lowest at low and high densities. In my work, from the range of the densities I encountered, based on the work of Allen *et al.* (1998) which has suggested that the density at which apostatic selection becomes anti-apostatic is about 131 $m^{-2}$, I assumed that the levels of density were as follow: low, <85 $m^{-2}$; intermediate, between 85-155 $m^{-2}$ and high, <155 $m^{-2}$. It is possible my estimation to the relative densities was different from that of Greenwood (1969). However, I have mentioned in section 5-1-4 that when one looks at the highest and the lowest values of diversity there seems to agree with the speculation of Greenwood (1969).

## *6-3- The significance of the effect of palatability and anti-apostatic selection*

Selection on distasteful prey should lead to monomorphism (Benson, 1972; Greenwood *et al.*, 1981; Allen, 1988a). Rare morphs of unpalatable species will be at a selective disadvantage and would have greater mortality than common morphs (Benson, 1972). This assumption depends on two reasons. For example, one would expect that inexperienced predators will have to eat about the same number of the two sorts of distasteful dimorphic prey before learning that they are distasteful and therefore will select proportionally more of the rare forms than the common ones. Furthermore, if there is a competition among a variety of different predators then this might lead to each predator visiting in turn and picking one single prey as fast as possible. This may increase the tendency to take the rare form, which looks conspicuous against the common one (Allen, 1972; Greenwood, 1984, 1985; Church *et al*, 1996; Fernández-Jurcic *et al.*, 2001)

In these experiments selection on high density of prey was usually anti-apostatic, whether the prey were artificial or live and whether the birds were wild or captive. Two species (robins and house sparrows), a pair of blackbird individuals and a pair of robin individuals all showed anti-apostatic selection. However, there was no significant effect of palatability apart from the live prey experiments which could mean that birds sometimes respond differently to live prey, especially if they are moving. In addition, the effect of palatability was significant when selection by the two individual blackbirds was examined separately, but it was a result of the strong preference for red when prey were palatable and not distasteful.

Ohguchi (1981) stated that the advantage of the "confusion effect" is dependent on the prey being monomorphic. He found that after dying all water fleas red, the stickleback *Gasterosteus aculeatus* took longer to capture any one of them. His finding supports the idea of Kruuk (1972) who concluded from his work on African cats and their prey that the presences of a few morphologically distinctive individuals in a prey group improves the predator's capture success. Both these experiments involved predators chasing their prey. Thus the results of the live prey experiments that showed a significant effect of palatability on frequency-dependent selection might have been influenced by the mobility of the prey, which in other experiments with maggots known to enhance anti-apostatic selection

(Wilson *et al.*, 1990). It would be interesting to design an experiment similar to that of Wilson *et al.* (1990) but this time with distasteful prey to investigate if there was an interaction between movement and palatability.

The strong anti-apostatic on distasteful prey in the fixed palatability design might have been caused by the birds feeding on the distasteful maggots, and learning to associate distastefulness with the colour of the rare form, assuming that they picked the rare colour first. When the frequency was altered the birds tended to avoid this same colour, which was now common, and concentrated on the rare type. The same argument might be applied to Greenwood *et al.*'s (1981) experiment with chicks *Gallus gallus* which showed that the chicks fed apostatically when they were given a distasteful food. They attributed this to variation in hunger between individual predators which may mean that while some deliberately fed on the distasteful food and selected apostatically, others sampled it while learning it was distasteful and so selected anti-apostatically. It might be that the chicks fed on the rare form first, because it stood out conspicuously against the common form (Allen, 1972; Greenwood *et al.*, 1984a, 1984b; Church *et al.*, 1996), and thus they were able to associate its colour with distastefulness and thus avoided it in the subsequent trials. Lindström *et al.* (2001) found that the great tits *Parus major* attacked the aposematic rare form disproportionately less in day 2 after learning to associate its square symbol with distastefulness.

Therefore, my argument is that at high density of distasteful prey, a predator may sometimes be able to learn to avoid the colour of the rare distasteful form. Kapan (2001), testing for the unexpected polymorphism in the unpalatable butterfly *Heliconius cydno*, has provided some support for this argument. In Ecuador two morphs (yellow and white) of this butterfly resemble two different unpalatable species *H. eleuchia* (yellow) and *H. sapho* (white). Kapan tested the prediction that differences in survival between experimental (the rare form) and control (the common form because it matches the colour of the co-model) butterflies decreases with increasing release densities. He captured pairs of yellow and white *H. cyndo* and released them at four sites that were dominated by yellow or white co-model species. In three sites the releasing density was low (1 pair every 163-194 m of trial) while in one site the releasing density was high (1 pair every 40 m of trial). The results of this experiment demonstrated that at a high releasing density there were no life-expectancy differences between experimental and control butterflies.

Thus these results lend further support to my argument. However, anti-apostatic selection might still occur due to variation among and within predation by different species (Allen & Anderson, 1984; Sherratt & MacDougall, 1995). Even if the same individual is used in the experiment, heterogeneity may result since a predator's behaviour in later trials may depend on its experience in earlier trials. For example, Horsley *et al.* (1979) pointed out that selection exerted by experienced birds (i.e. in the second halves of the experiments), differed from that exerted by naïve birds (i.e. in the first halves of the experiments). The experiments in chapter 3 demonstrated such variation. The direction of selection by blackbirds, unlike the other three target species, was against the common form and their colour preference seemed to be towards yellow. Yet at the individual level, a mated pair of blackbirds selected against the rare form as a result of palatability which might have affected their strong preferences for red. Variation was also apparent between two individual robins of which one of them had strong preference for the rare form while the other had strong preference for yellow (as well as a preference for the rare form). Similarly, captive Fisher's lovebirds showed no selection when feeding on the distasteful baits, but overall, selection by all captive birds was anti-apostatic on both the palatable and distasteful baits.

## 6-4- Frequency-dependent selection at high densities

The effect of palatability was insignificant in the artificial prey experiments, which contradicts the results of Greenwood *et al.* (1989) who found that the strength of anti-apostatic selection is affected by palatability. However, my results showed that like feeding on palatable prey at high density (Allen, 1972; Ohguchi, 1978, 1981; Allen & Anderson, 1984; Greenwood *et al.*, 1984; Allen *et al.*, 1998; Weale *et al.*, 2000), birds still consumed distasteful prey in an anti-apostatic manner. Greenwood (1984) has suggested that the explanation for anti-apostatic selection occurring at high densities but not contiguous might be that animals may be confused by the presence of more than one prey item in their sensory field at any one time. Thus it seems probable that an animal will be more efficient if it attends to prey that differ from all the other prey that are in its sensory field than if it tries to attend to individuals that are similar to many of the others that are present. Another theory is that the predators might test unfamiliar prey for toxicity or unpalatability in this sense by taken small amount of each type. If the small amount taken from two types of prey were roughly equal, the predator would appear to have preference for the rare form (Greenwood, 1985). Whatever the reason we would therefore predict that prey populations should be monomorphic at very high densities irrespective of palatability. Yet the investigation in chapter 5 showed that diversity of *Umbonium vestiarium* seemed not to be affected by anti-apostatic selection. This might be caused by the massive polymorphism with each morph different from its conspecific neighbours that visual predators find it hard to establish specific searching image for one morph (Moment, 1962, Allen, 1988b; Owen & Whiteley, 1988). Heterosematism could also be a factor that influences the direction of selection at high densities. From the experiment with pastry baits in chapter 5, heterosematism does indeed appear to increase diversity. Also one might speculate that the density at which visual predators encounter *U. vestiarium* is perhaps effectively low because this snail lives near low-water mark (Grüneberg, 1980) and it is mostly buried in the sand and thus predators would only see those snails that appeared at the surface when the water surf breaks.

## 6-5- Frequency-independent selection at high densities

In some experiments especially when live prey were used, I observed a high intake of the distasteful prey not much below that of the palatable. This may have been because the predators, when faced with a diversity of resources may forage so as to maximise energy gain and thus reduce the cost of hunting (Gianino & Jones, 1989). Such optimal foraging may lead to a concentration on certain prey items or particular foraging sites at the expense of others (Stephens & Kerbs, 1986) or even certain colours. This could explain why, overall, most of the experiments showed that the birds exhibited some preferences for one particular colour, usually yellow. Wilson *et al.* (1990) also noticed that wild birds feeding on the high prey density preferred the yellow maggots over the red ones. In the preliminary experiment in chapter 4 wild birds preferred the bronze water-dipped maggots, which is close to yellow in term of hues, over red water-dipped maggots. On the other hand, Greenwood *et al.* (1981) found that birds had a strong preference for red over yellow and Greenwood *et al.* (1989) found no significant effect of frequency-independent selection on their yellow and red baits. However, the density at which they presented the red and yellow baits for was low.

It might also be that the birds had an established aversion to red (Sillén-Tullberg, 1985b; Roper, 1990). This aversion has been noticed also in experiments with mimicry, Morrell & Turner (1970) found that wild birds strongly avoided red mimics compared yellow mimics; however the model was also red. Goodale & Senddon (1977) found that the use of red models resulted in a significantly less predation on mimetic pastries than did the use of yellow models. In an experiment using brightly coloured pastry prey, Raymond (1987) also observed that birds tended to avoid red coloured baits. She attributed this behaviour to past experiences with aposematic species such as the ladybird *Adalia bipunctata*. The captive birds in my experiment showed no colour preference, perhaps because they had had no experience with aposematic species. Furthermore, in the experiment with heterosematic pastry prey, birds showed preferences for red in the first presentation but preferred yellow in the second presentation, implying that red was more aversive when novel.

As in many experiments that used high density of green and brown pastry baits (Allen, 1972; Horsley *et al.*, 1979; Allen *et al.*, 1998; Weale *et al.*, 2000) birds preferred brown over green. Turček (1963), testing the colour preferences of 156 species of birds in Europe, found that brown is among the favourite colours, perhaps because brown diaspores are abundant in Europe. Green is among the less preferred colours and Turček claimed that this colour is not attractive to birds and is thus selectively disadvantageous. Preference for brown has also been observed at low density 'bait' experiments (Allen & Clarke, 1968; Allen, 1974, 1976). Thus, perhaps, birds usually acquire preferences for brown over green irrespective to density.

## 6-6- Suggestions for future work

The results of my experiments have shed light on other areas for future work. Experiments with known free-ranging wild bird species in chapter 3 have shown that selection by blackbirds, unlike selection by the other three species (robins, house sparrows and blue tits) was in the direction of apostatic selection. In this experiment blackbirds on many occasions attended some of the first trials at a given site before either never reappearing or visiting erratically. This particular behaviour might have caused the data obtained from studying them to be very variable. Thus there is a need for more replications with more individuals of blackbird.

The two experiments with live maggots in chapter 4, unlike experiments with pastry baits, have demonstrated that palatability does influence frequency-dependent selection. Perhaps, the movement of the prey might have accelerated the avoidance learning by predators. Thus it would be interesting to design an experiment similar to the experiment of Wilson *et al.* (1990) but this time with distasteful prey to investigate whether there is a significant interaction between palatability and movement.

The field survey designed to explore whether *Umbonium vestiarium* is less polymorphic (i.e. whether anti-apostatic selection is acting) at high densities failed to demonstrate a significant interaction between density and diversity. However, there is a suggestion of a positive relationship of diversity and density (e.g. at Ras Al-Hid, fig 5-5). Thus there is a case for sampling many more populations of *U. vestiarium.*

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