This is a pre-copyedited, author-produced PDF of an article accepted for publication in Oecologia following peer review. The final publication is available at Springer via http://dx.doi.org/10.1007/s00442-012-2473-γ

Mixed phenotype grouping: the interaction between oddity and crypsis

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Author contributions: GMR, HK and LJM conceived and designed the experiments. GMR and HK performed the experiments. GMR, HK and LJM analysed the data. GMR and LJM wrote the manuscript.
Abstract

Aggregations of different-looking animals are frequently seen in nature, despite well-documented selection pressures on individuals to maintain phenotypically homogenous groups. Two well-known theories, the ‘confusion effect’ (reduced ability of a predator to accurately target an individual in a group) and the ‘oddity effect’ (preferential targeting of phenotypically distinct, ‘odd’, individuals) act together to predict the evolution of behaviours in prey that lead to groups of animals that are homogeneous in appearance. In contrast, a recently-proposed mechanism suggests that mixed groups could be maintained if one species in a mixed group is more conspicuous against the habitat than the other, as confusion effects generated by the conspicuous species impede predator targeting of the cryptic species; thus, cryptic species benefit from association with conspicuous ones. We test these contrasting predictions from the perspective of both predators and prey, and show that cryptic individual Daphnia are at reduced risk of predation from three-spine sticklebacks Gasterosteus aculeatus when in mixed phenotype groups, a risk that is reduced further as the number of conspicuous individuals increases, supporting the hypothesis for the evolution of mixed groups. In contrast, while the preference for associating with colour-matched conspecifics by mollies (Poecilia sphenops) was reduced when they were cryptic, we found no evidence for active association with conspicuous conspecifics. We conclude that prey animals must balance the relative risks of oddity and conspicuousness in their social decisions, and that this could potentially lead to the evolution of mixed phenotype grouping as a response to predation risk alone.

Keywords

Mixed-species group, inter-specific grouping, oddity effect, confusion effect, predation
Introduction

Predators play a key role in ecological communities with well-established direct and indirect effects on prey abundance and behaviour (Sih et al. 1985; Lima and Dill 1990; Schmitz et al. 2004). Theoretical understanding of predator-prey interactions is generally based on the assumption of homogeneous, randomly distributed prey. For example, although functional response models (predicting predation success in response to prey density) have been developed that account for handling and digesting time of predators, such models assume spatial and phenotypic homogeneity in prey (Jeschke et al. 2002). Other theoretical approaches in ecology that include these assumptions include population dynamics (Tenhumberg et al. 2009), group formation (Morrell et al. 2011) and biological self-organisation (Couzin et al. 2005). The assumptions of prey homogeneity can be violated in two important ways: 1) a violation of the assumption of spatial homogeneity via prey aggregation and 2) a violation of the assumption of phenotypic homogeneity where prey differ in appearance or behaviour. Including these factors into models of predator-prey interactions can have significant effects on their predictions (Fryxell et al. 2007; Pettorelli et al 2011).

Aggregation is a widespread phenomenon across the animal kingdom carrying with it numerous benefits for individuals living in groups (Krause & Ruxton 2002). Perhaps the most well recognised benefits relate to a reduction in predation risk through several mechanisms including the dilution (Foster and Treherne 1981), encounter-dilution (Turner and Pitcher 1986), selfish herd (Hamilton 1971) and confusion (Miller 1922; Krakauer 1995) effects. The confusion effect describes the inability of a predator to accurately target individual prey items within a group of moving individuals, resulting in a reduced attack-to-kill ratio and
benefiting all individuals in the group (Krakauer 1995; Krause & Ruxton 2002). This effect is predicted to be enhanced by increased synchrony of movement, larger group size, increased density and phenotypic uniformity within a group. Where there is variation in phenotypes in a group, predators can increase their success rate if they select prey that are phenotypically distinct from the rest of the group. This is known as the oddity effect, and results in preferential predation on ‘odd’-looking individuals (Milinski 1977; Ohguchi 1978; Krause & Ruxton 2002). Predator confusion and the oddity effect are complementary mechanisms that select for behaviours in prey that should result in the formation of phenotypically-assorted (homogeneous) groups. In systems where the predator hunts visually prey groups should consist of individuals that are all very similar in appearance, and evidence suggests that this is often the case. Shoaling fish have been well-studied in this regard where assortment by species (Ward et al. 2002), body size (Krause et al. 1996), kinship (Fitzgerald and Morrissette 1992), parasite load (Barber et al. 1998) and colour (Rodgers et al. 2011) are observed.

However, there are many cases where there is considerable variation in phenotype within a group. This is particularly clear in communities where mixed-species grouping occurs. Mixed-species associations are frequently observed in birds (Moynihan 1968), mammals (Smith et al. 2004), and fish (Barlow 1974). Although mixed-species grouping has been the subject of scientific investigation for over 100 years (Morse 1977) we do not fully understand the evolutionary causes and the mechanisms by which it is maintained in the face of selection for phenotypic assortment in groups (via the confusion and oddity effects; Tosh et al. 2007). A number of possible benefits of associating with individuals that are not reliant on phenotype-matching (i.e. not linked to avoiding oddity) have been described
which may explain the occurrence of mixed-species and mixed-phenotype groups, including increased foraging efficiency and predator detection for all group members (Stensland et al. 2003). Alternatively, the benefits of mixed species grouping may be asymmetric.

Experimental evidence suggests that a solitary individual may benefit from joining a group of dissimilar con- or heterospecifics rather than remaining alone (Landeau and Terborgh 1986), may preferentially associate with more vulnerable individuals (Mathis and Chivers 2003) or with ones better able to detect predators (Diamond 1981; Krause and Ruxton 2002). This active choice by some group members means that the costs of oddity may be greater for some individuals in a group than for others (Mathis and Chivers 2003; Rodgers et al. 2011).

Using neural network models Tosh et al. (2007) proposed a predation-based mechanism to explain mixed-species grouping. They introduce the idea that the interaction between crypsis, confusion and oddity can lead to the evolution of mixed species grouping. Here crypsis (or conspicuousness) refers to the animal’s colouration relative to the habitat, contrasting with oddity, which refers to colouration relative to the rest of the group. Tosh et al. (2007) demonstrate theoretically that when groups consist of both cryptic and conspicuous individuals, confusion effects generated by the conspicuous group members are of particular benefit to cryptic individuals. They suggest that the anti-predator benefits of crypsis (Ruxton et al. 2004; Caro 2005) may be enhanced by association with conspicuous species as this worsens predator targeting of the cryptic individuals beyond that predicted by either their crypsis alone or by the confusion effect, but there is no experimental evidence to confirm this.
Both the mechanism presented in Tosh et al. (2007) and the standard confusion/oddity framework described above predict asymmetric (phenotype dependent) predation risk on prey, but these predictions differ dependent upon whether the prey animals are conspicuous or cryptic relative to the habitat. Table 1 outlines the contrasting predictions for the two possible mechanisms, which can be summarised as follows: Tosh et al. (2007) predict that phenotypically distinct (odd) individuals should only be targeted if they are conspicuous, and that individuals that are both cryptic against the habitat and odd relative to the group are targeted less often than expected by chance. In contrast the confusion/oddity framework predicts that odd individuals should always be targeted, regardless of their crypsis against the habitat (table 1a). From the perspective of the prey (table 1b), the confusion/oddity framework predicts that individuals should preferentially associate with colour-matched group-mates (leading to the evolution of homogeneous groups), while Tosh et al (2007) predict that this should only be true for individuals that are conspicuous against the habitat; cryptic individuals should choose to associate with conspicuous (and therefore phenotypically different from themselves) rather than colour-matched group-mates (table 1b), potentially leading to the evolution of mixed grouping.

We examine these hypotheses from the perspective of both predators (three-spined sticklebacks *Gasterosteus aculeatus* attacking individuals in groups of colour dyed *Daphnia*) and prey (black and white morphs of the molly *Poecilia sphenops* choosing to shoal with matched or dissimilarly coloured shoal mates). By exploring both the predator and prey perspectives our work will more fully explore the various pressures that shape predator-prey interactions and lead to the evolution of the mixed-phenotype groups of prey animals we see in nature. Our aim is to explore patterns that apply broadly to groups of prey
animals by using two different model systems to address the prey and predator angles of this phenomenon. Each system was selected to be the most suitable available to us for those particular experiments. This integrated approach allows a greater understanding of the mechanisms involved in the evolution and maintenance of mixed-phenotype aggregations.

Materials and methods

Experiment 1: Prey targeting by sticklebacks

Approximately 150 three-spine sticklebacks (Gasterosteus aculeatus) were collected from Saltfleet, Lincolnshire in October 2009 and housed in large opaque containers at a temperature of 15°C under a 12:12hr light:dark cycle. Fish were fed daily on frozen bloodworm. Live Daphnia magna were obtained from a local pet shop. To obtain cryptic and conspicuous prey, live Daphnia were dyed red or blue by placing large numbers of individuals in 1000ml of water containing 5ml of either red or blue food dye (Dr. Oetker brand) for a period of 7-10 days. Red and blue were chosen as both colours are present in stickleback breeding colouration and there is evidence that these fish rank shades of these colours in a similar way to humans (Rowe et al. 2006). Blue food colouring contained: water, colour (brilliant blue), acidity regulator (citric acid) and preservative (potassium sorbate). Red food colouring contained: colours (beetroot red, paprika extract), mylose syrup, emulsifier (polysorbate 80), glycerine, water, antioxidants (disodium EDTA, sodium ascorbate) and preservative (potassium sorbate).

Prey targeting experiments were carried out in a small aquarium (20x20x50cm). The tank was divided into two sections: a horizontal wire positioned on the base of the tank marked
out the third of the tank furthest from the prey (the predator zone). At the opposite end of the tank, a removable grid of 1cm transparent cubes positioned on the external wall of the tank held the *Daphnia*. Twelve cubes were arranged in a 3x4 grid and placed centrally on the outside of the tank end. Each cube contained coloured water (red or blue at 3ml/litre) and a single *Daphnia*. The concentration of food dye in the cubes was slightly lower than that used to dye the *Daphnia* to ensure that individual *Daphnia* were visible to the observer, while enhancing crypsis where water and Daphnia were the same colour. The grid design ensured that the 12 Daphnia prey were unable to aggregate, as predators are known to target denser areas of groups (Ioannou et al. 2009). Inside the tank, flush with the wall containing the prey, we positioned a removable opaque barrier to conceal the prey from the predator during an acclimatisation period. The tank was surrounded by opaque screens to minimise disturbance to the fish. Trials were recorded using a digital video camera (Panasonic NV-GS280) placed behind the grid containing the prey, such that the predator was viewed through the prey grid and the prey item targeted could be easily identified.

Each section of the grid contained dyed water and a single *Daphnia*. We considered 4 primary treatments:

1) Control: 12 undyed *Daphnia* in either red or blue water, to test for any innate preference for red or blue (20 trials)

2) 6:6 treatment (even treatment): equal numbers of red and blue dyed Daphnia (40 trials)

3) 1:11 treatment (cryptic minority): a single cryptic individual and 11 conspicuous individuals (40 trials)
4) 11:1 treatment (cryptic majority): 11 cryptic individuals and a single conspicuous
individual (40 trials)

The specific predictions for each experiment in relation to our hypotheses (table 1a) can be
found in table 2a. For treatment 1 (control), 6 cells contained red-dyed water and 6 cells
contained blue-dyed water arranged such that the immediate neighbours of any cell were of
the opposite colour. Treatments 2-4 had a single colour background (i.e. all cells contained
red or blue water) and were repeated with both red and blue backgrounds (N=20 for each
colour background). Cryptic individuals were those that were the same colour as the
background, conspicuous ones were those that were the opposite colour. In treatment 2,
individual *Daphnia* were positioned so that the direct neighbours of each individual was of
the opposite colour to avoid any clustering of particular phenotypes. In treatments 3 and 4
the position of the odd individual was changed systematically between trials to control for
any centre or edge preferences in the attacking predator. Once filled, the prey grid was
positioned externally on the tank. *Daphnia* were taken from pools of similarly coloured
individuals to which they were returned between trials. Pools contained approximately 50
*Daphnia* of 3mm +/- 0.25mm.

An individual stickleback was placed into the predator zone in the test tank and given 2
minutes to acclimatise. After the acclimatisation period we raised the opaque barrier
concealing the prey, using a pulley to reduce disturbance to the fish. If the stickleback was
not in the predator zone, the barrier was raised once the fish returned there. We recorded
the colour and crypsis of the first prey individual attacked by the fish, using the video
recordings. Fish that did not enter the predator zone within 10 minutes were removed. Fish
that did not attack the prey within 15 minutes of the barrier being removed were excluded
from the analysis. Final sample sizes were \( N = 16 \) (control; 4 fish did not attack), \( N = 40 \) (even treatment; all fish attacked), \( N = 38 \) (cryptic minority; 2 fish did not attack) and \( N = 38 \) (cryptic majority; 2 fish did not attack)). Fish were not reused and the water in the test tank was changed between trials.

Experiment 2: Shoal choice in mollies

Mollies (\textit{Poecilia sphenops}) occur in two distinct colour morphs (black and white) but are phenotypically similar in other aspects of morphology. 70 black and 70 white individuals were obtained from Neil Hardy Aquatica in December 2009 and maintained in small aquaria (200x200x500mm) with a gravel substrate, small filter and artificial plant, at a salinity of 1.004ppt at 26\( ^\circ \)C and on a 12:12hr light:dark cycle. Each tank contained 10-12 individuals, with equal numbers of each morph. On arrival in the laboratory, fish were randomly assigned to be either test fish (approximately 60 individuals of each colour) or in the initial pool of stimulus fish (10 individuals of each colour; see also below). Test and stimulus fish were held separately, and within a category (test/stimulus) individuals were moved between tanks twice a week for the duration of the experiment to reduce any confounding effects of familiarity (Griffiths 1997; Griffiths and Magurran 1997a). Fish were housed in these conditions for approximately 6 weeks until commencement of the trials, and were fed commercial fish food twice daily.

Shoal choice experiments were carried out in 2 test aquaria. Each tank (200x500x170mm) was divided into 3 sections using transparent glass to ensure visual but not olfactory communication to reduce confounding effects of habitat similarity (Webster et al. 2007). The two end compartments (stimulus compartments) measured 150x200mm and contained
the stimulus fish. Three of the external surfaces of each tank were covered with opaque
adhesive film, leaving only the side facing the observer transparent. One tank was covered
with white film, the other with black film. A 20mm layer of white or black gravel respectively
was also added. By performing the trial in either a black tank or a white one, each of the fish
morphs could be made cryptic or conspicuous against the background. Preference zones
were marked on the observer side of the central compartment at a distance of 82mm (twice
the mean body length of 20 fish) from the stimulus compartments. A test fish was
considered to be shoaling with a stimulus shoal when more than 50% of its body was in the
preference zone, giving a conservative estimate of shoaling tendency.

Only fish with a standard body length of between 38 and 46mm were used in the
experiment, and there was no significant difference in body size between black and white
fish ($F_{1,106} = 1.845, p = 0.177$). 4 black individuals were taken from the pool of stimulus fish
and placed in one stimulus compartment and 4 white individuals were placed in the other
stimulus compartment. A test fish was placed in the central compartment and allowed 10
minutes to acclimatise before preferences were recorded. We recorded the cumulative time
(in seconds) that the fish spent in each of the preference zones over the course of a 10-
minute trial. All 4 combinations of test fish colour and crypsis (background colour) were
investigated. The specific predictions for each combination in relation to our hypotheses are
outlined in table 2b. The stimulus compartment containing the black fish was alternated
between experiments to control for side bias. Once fish had been used as test fish they were
added to the pool of stimulus fish, but stimulus fish were never used as test fish. 30 trials
were conducted where the test fish was white and cryptic (i.e. a white fish on a white
background), 30 on white conspicuous fish, 28 on black cryptic fish and 26 on black conspicuous.

Statistical analysis

Binomial tests were used to investigate the prey selection preference of sticklebacks. Expected proportions are given in the relevant section below. For the shoaling experiment we used a generalised linear model with quasi-binomial error distribution (to account for overdispersion in the data) and a logistic link function to investigate the proportion of time spent shoaling with the colour-matched shoal as a function of test fish colour and test fish crypsis. The interaction between test fish colour and test fish crypsis was not significant and so was removed to give the minimum adequate model. To investigate whether the shoaling preference exhibited by each colour/crypsis combination differed significantly from a random preference, we tested (preference for matched shoal)-(preference for unmatched shoal) against a null expectation of zero using one-sample t-tests. Data were arcsin square root transformed to meet the assumptions of normality. Correction for multiple tests was carried out using False Discovery Rate control (Benjamini & Hochberg 1995). Both original and adjusted (in italics) p-values are shown.

Results

Experiment 1: Prey targeting in sticklebacks

There was no colour preference for undyed Daphnia on red or blue backgrounds (binomial test, \( P = 1.00 \) (\( P=1.00 \))) and no difference between colour treatments (i.e. whether the trials were performed on a red or blue background) for any of the ratios tested (binomial tests...
1:11 P = 1.00 (P = 1.00); 6:6, P = 0.44 (P = 0.572); 11:1, P = 0.45 (P = 0.532)). Therefore all data have been pooled and analysed on the basis of cryptic/conspicuous alone.

The number of times the cryptic individual was targeted by the predator was significantly lower than random expectation in the 6:6 (cryptic individual targeted in 7/40 trials, against a random expectation of 0.5, P < 0.001 (P < 0.001)) and 11:1 experiments (cryptic individual targeted in 26/38 trials, against an expectation of 0.9167 (11/12), P < 0.001 (P < 0.001)), but not in the 1:11 experiment (cryptic individual targeted in 1/38 trials, against a random expectation of 0.0833 (1/12), P = 0.370 (p = 0.535), figure 1a). Thus, cryptic individuals are targeted less often than expected by chance when grouped with a lower or equal number of conspicuous individuals. When a single cryptic individual is in a group with predominantly conspicuous individuals, it is attacked at a rate consistent with random attack. Table 2a summarises these results in relation to the specific predictions of both the Tosh and oddity mechanisms.

We next investigated the per capita predation risk for cryptic and conspicuous individuals for each of the group compositions tested (figure 1b). In a group consisting entirely of one type or the other (N = 12 in figure 1b), the per capita risk for each individual is 0.0833 (=1/12). We calculated the per capita risk for each composition as the proportion of trials in which an individual of the type under consideration was targeted divided by the number of individuals of that type in that trial. Thus, for the 1:11 treatment, per capita risk for cryptic individuals was calculated as (1/38)/1 (one cryptic target in 38 trials, with 1 cryptic individual in the trial) and risk for conspicuous individuals as (37/38)/11 (37 conspicuous targets in 38 trials divided by the 11 cryptic individuals in each trial). Per capita risk for cryptic individuals
in greatest when in a uniform group and decreases as the number of conspicuous individuals in the group increases. In contrast, per capita risk for conspicuous individuals is lowest in a uniform group and increases with the number of cryptic group-mates (figure 1b).

**Experiment 2: Shoal choice in mollies**

Fish colour and fish crypsis had significant independent effects on the proportion of time spent shoaling with the colour-matched shoal (quasi-binomial GLM, colour: $t = 2.861, df = 106, P = 0.0038 (P = 0.0083)$, crypsis: $t = -2.320, df = 105, P = 0.0197 (P = 0.0366)$, non-significant interaction between crypsis and colour removed from the model). Conspicuous fish showed a stronger preference for the colour-matched shoal than cryptic fish and white fish showed a stronger preference than black fish (figure 2). Preference for the colour matched shoal differed significantly from random choice for white conspicuous fish ($t = 7.4733, df = 28, P < 0.001 (P < 0.001)$), black conspicuous fish ($t = 4.3172, df = 25, P < 0.001 (P < 0.001)$), and white cryptic fish ($t = 6.3823, df. = 26, P < 0.001 (P < 0.001)$) but not for black cryptic fish ($t = 1.2425, df = 25, P = 0.2256 (P=0.3666)$). Table 2b summarises these results. There was no difference in overall shoaling tendency between cryptic and conspicuous test fish (t-test, $t = 0.201, P = 0.841$).

**Discussion**

The two theoretical frameworks for the evolution of mixed phenotype groups that we have investigated predict different suites of predator targeting of prey, prey risk and prey group choice behaviour. The confusion/oddity framework (preferential predation on phenotypically distinct, odd individuals in a group) selects against the evolution of mixed grouping, but mixed aggregations are selected for by the mechanism proposed in Tosh et al
Here, grouping is driven primarily by one partner in a species/phenotype pair, as the confusion effect generated by a species that is conspicuous against the habitat impedes predator targeting of a more cryptic one. Our results (summarised in table 2) lend support to both mechanisms and suggest that animals may face conflicting selection pressures within the context of phenotypic similarity in group assortment. We show that individuals that are cryptic against the habitat are at reduced risk of predation when in mixed phenotype groups and this risk is reduced further as the number of individuals that are conspicuous against the habitat increases, providing support for Tosh’s model. In contrast, and in support of the confusion/oddity framework, we did not find any evidence that cryptic fish preferentially chose to associate with conspicuous ones. Instead, in the majority of our tests, fish associated with phenotypically similar individuals, avoiding being odd in the group, but this preference was reduced when individuals were cryptic against the habitat.

We provide evidence for asymmetric costs to individuals in mixed groups: in our prey targeting experiment, cryptic individuals benefitted by association with conspicuous ones while conspicuous ones were put at increased risk by the presence of cryptic individuals (figure 1b). As Tosh et al (2007) suggest, mixed grouping could therefore be maintained by the association preferences of cryptic individuals (or those that are simply at lower risk of predation). There are many examples of associations in mixed species groups being maintained by one party: associations between fathead minnows (*Pimephales promelas*) and brook sticklebacks (*Culaea inconstans*) are maintained by the less vulnerable sticklebacks (Mathis and Chivers 2003), and cowtail stingrays (*Pastinachus sephen*) maintain the association with whiprays (*Himantura uarnak*) because of the whiprays faster antipredator response (Semeniuk and Dill 2006). Similar patterns are seen within species: in
European minnows (*Phoxinus phoxinus*), good foraging competitors choose to actively associate with poor competitors but not vice versa (Metcalfe and Thomson 1995). The results of our shoal choice experiment, however, do not support the idea that cryptic individuals preferentially associate with conspicuous ones; instead suggesting that crypsis allows more flexibility in shoaling decisions.

The context and visual background in which prey animals are being observed is important when considering crypsis (Endler 1990). When a predator is at some distance from prey, crypsis against the background (e.g. vegetation or substrate) is likely to be of primary importance in concealing a prey group. As predators must identify and then select a group to attack, this would select for all group members to match their background (Ruxton et al. 2004) producing phenotypically uniform groups. At closer range, once the group has been detected, predator focus switches to identifying and targeting an individual within a group. Relative risks within the group become important and behaviours that reduce an individual’s risk relative to his group mates are selected for (Morrell et al. 2011). Our findings from the shoal choice experiments may represent a trade-off between reducing risk pre- and post-detection.

Conflicting selection pressures in social decision-making are not uncommon. The decision to join one group over another depends on many more factors than phenotypic appearance alone. Group size (Krakauer 1995), nutritional state (Krause 1993a; Morrell et al. 2007), parasitism (Barber and Huntingford 1995), predation risk (Hoare et al. 2004), familiarity (Griffiths and Magurran 1997b) and recent experience (Webster et al. 2007) all interact to shape shoaling decisions. Membership of a larger group, for example, may benefit
individuals through the dilution effect (Foster and Treherne 1981; Turner and Pitcher 1986), but this must be traded off against the relative ease of detection of larger groups by predators (Ioannou and Krause 2008; Morrell and James 2008) and the importance of familiarity in shoal choice decisions decreases as group size increases (Griffiths and Magurran 1997b).

There may be other pressures selecting for phenotypic uniformity in groups, including activity synchrony (Conradt and Roper 2000) and foraging efficiency (Ranta et al. 1994). Conradt and Roper (2001) propose that uniformity is maintained by the higher cost of performing synchronous activities for mixed groups, while Ranta et al. (1994) suggest that foraging success should be higher in uniform groups, particularly for small individuals. In addition, there may be social pressure to maintain uniform groups. One can imagine that high-risk (here, conspicuous) individuals would benefit by ‘evicting’ low-risk (cryptic) ones from their group. There is little evidence that individuals can control group membership in ‘free entry’ groups such as fish shoals (Krause and Ruxton 2002), but where groups are stable and social hierarchies exist, entry to a group may be restricted (Stephens et al. 2005; Jordan et al 2010). Even in free entry groups, less favoured group members may be restricted to the periphery (Krause and Godin 1994; Barber et al. 2000) where predation risk is higher (Hamilton 1971; Krause 1993b). Alternatively, high-risk individuals made vulnerable by new low-risk members could choose to leave the shoal when the risks associated with leaving are outweighed by the risks imposed by non-uniformity of the group.

It is possible that the animals used in our experiments did not perceive colour differences in the same way as the human observers. However, sticklebacks are known to rank red and
blue in a similar way to humans (Rowe et al. 2006) and so individuals that appeared cryptic
and conspicuous to us are likely to appear similarly to the fish. Little is known about colour
perception in mollies, but previous work suggests a perception of black and white that is
consistent with ours (Bradner and McRobert 2001). We found that black fish generally
showed a weaker preference for the colour-matched shoal, but this reflects previous
findings (McRobert and Bradner 1998) and may result from a reduced perception of risk by
black fish (perhaps due to an increased perception of crypsis or safety) or selected
differences in shoaling preferences resulting from domestication.

We acknowledge the limitations of using captive-bred, domestic animals in some of our
experiments. Captive-bred animals have not been subject to the selection pressures which
their wild counterparts experience, and in the case of the mollies, the captive breeding and
selection regimes necessary to produce distinct black and white morphs likely means that
black fish are more closely related to other black fish than to white fish and vice-versa. This
potentially confounds any effect of colour with that of kinship, by which fish are also known
to assort (Krause and Ruxton 2002; Ward & Hart 2003). However, while our finding that fish
preferentially associate with similarly coloured individuals could be explained as a
preference for associating with more closely related individuals, the key finding is that
preferences change according to the conspicuousness of the potential shoal-mates. This
suggests that there are other colour-associated factors at play in determining shoal choice in
mollies. We suggest that our results demonstrate association patterns based on colour,
oddity and crypsis that go beyond the confounding effects of relatedness resulting from
domestication.
We chose to use black and white mollies because of their very similar morphologies, distinctly different colours, their history of use in similar experiments and their documented ability to discriminate between different colour morphs and associate on the basis of colour, with variation in preference strength depending on the characteristics of the shoal and environment (McRobert and Bradner 1998, Bradner and McRobert 2001a, b). These studies are often cited as the classic examples showing that fish assort by colour and their authors have suggested that shoal-choice abilities and preferences for particular phenotypes in shoal-mates is so important to group living fishes it is likely to be highly conserved and still present even in domestic morphs (Bradner and McRobert 2001a). Domestic morphs of group-living fishes have been successfully used to demonstrate and explain patterns of association and social learning seen in wild animals (Laland and Williams 1998; Reader and Laland 2000; Engeszer et al. 2004; Morrell et al. 2007; Gomez-Laplaza 2009). Examples of the colour assortment seen here in domestic fish reflect those seen in wild fishes (Crook 1999; Rodgers et al. 2010).

The oddity effect is predicted to operate most strongly in small, highly asymmetric groups (Krause and Ruxton 2002), the conditions tested here. We also investigate oddity in two very different taxonomic groups on which different selection pressures may operate, resulting in different patterns. Further work is needed to elucidate the conditions under which oddity effects shape animal aggregations in nature, and the effects of interactions between oddity and other selection pressures (including crypsis) across species. The majority of work investigating the oddity effect in relation to colour has used either domesticated morphs (McRobert and Bradner 1998; Bradner and McRobert 2001; Gomez-Laplaza 2009) or artificially dyed prey (Ohguchi 1978; Landeau and Terborgh 1986; Thomas
et al. 2010) and future work should also consider natural variation in prey colouration, on which predators must base their choice of target and prey base their social decisions.

When confusion effects associated with aggregation are incorporated into functional response models, they significantly alter predicted rates of consumption relative to prey density (Jeschke and Tollrian 2005). Similarly, considering groups as a functional unit in models of predator-prey dynamics fundamentally alters predicted food intake rates and stabilises interactions (Fryxell et al. 2007). Thus, the processes and patterns involved in the formation and maintenance of animal groups are a key component in predator-prey interactions and the structure of animal communities. Variation between individuals is also thought to influence predator-prey dynamics (Petorelli et al. 2001): understanding how predators select from among available prey types may have implications for concepts ranging from the evolution of aggregation (Couzin et al. 2005; Morrell et al. 2011) and aposematic colouration (Ruxton et al. 2004) to understanding species diversity (for example, if predators preferentially consume rare prey species resulting in local extinctions; Almany et al. 2007). Here we show that prey animals must balance the relative risks of oddity and conspicuousness in their group choices and suggest that the complex selection pressures enforced by predation can lead to the evolution of mixed-phenotype grouping through response to these risks alone.

Acknowledgements

We thank Colin Tosh for invaluable discussions in the early stages of this project, Scott Fawcett and the CBS staff practical advice and fish husbandry services, Ása Johannesen for help with collection of fish, and Jennifer Kelley, Katherine Jones, Scott Peacor and two
anonymous referees for insightful comments on the manuscript. This work was funded through a Biotechnology and Biological Sciences Research Council Doctoral Training Grant to GMR, Natural Environment Research Council Postdoctoral Fellowship (NE/D008921/1) to LJM, and the University of Leeds (HK).

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### Table 1: Contrasting predictions of the confusion/oddity framework and the mechanism proposed by Tosh et al. (2007), for situations where a) predators are selecting from among available prey types, which may be phenotypically distinct from the majority of the group ('odd/rare' in the table) and may also be cryptic or conspicuous against the habitat; and b) when prey are selecting group-mates with whom to associate.

<table>
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<tr>
<th>Prey phenotype</th>
<th>Tosh et al. (2007) predict:</th>
<th>Confusion/oddity framework predicts:</th>
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<tr>
<td><strong>a) Predators selecting from among available prey types</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A prey animal which is:</td>
<td>Should be targeted:</td>
<td></td>
</tr>
<tr>
<td>Odd/rare relative to the rest of the group</td>
<td>When conspicuous against the habitat</td>
<td></td>
</tr>
<tr>
<td>Cryptic against the habitat</td>
<td>Less often than expected by chance when grouped with conspicuous individuals</td>
<td>When odd/rare</td>
</tr>
<tr>
<td>Conspicuous against the habitat</td>
<td>Always targeted</td>
<td>When odd/rare</td>
</tr>
<tr>
<td><strong>b) Association preferences of prey</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>An individual which is:</td>
<td>Should associate with a group which is:</td>
<td></td>
</tr>
<tr>
<td>Cryptic against the habitat</td>
<td>Conspicuous (and therefore of a different Cryptic (phenotypically matched to the choosing</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: A summary of our hypotheses relating to the predictions of the Tosh et al. (2007) model and the confusion/oddity framework, together with the qualitative results of the prey targeting (a) and shoal choice (b) experiments, indicating the model supported.

a) Prey targeting experiment

<table>
<thead>
<tr>
<th>Cryptic: conspicuous ratio</th>
<th>Prediction for cryptic individuals</th>
<th>Conflict</th>
<th>Result</th>
<th>Support for: Confusion/oddity predictions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tosh et al. (2007)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:11</td>
<td>Attacked</td>
<td>Attacked</td>
<td>Yes</td>
<td>Attacked at random</td>
</tr>
<tr>
<td></td>
<td>less than random</td>
<td>more than random</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6:6</td>
<td>Attacked</td>
<td>Attacked at random</td>
<td>Yes</td>
<td>Tosh</td>
</tr>
<tr>
<td></td>
<td>less than random</td>
<td>random</td>
<td>less than</td>
<td></td>
</tr>
<tr>
<td></td>
<td>random</td>
<td></td>
<td>random</td>
<td></td>
</tr>
</tbody>
</table>
### b) Shoal choice experiment

<table>
<thead>
<tr>
<th>Focal fish</th>
<th>Shoaling preference prediction</th>
<th>Conflict between predictions?</th>
<th>Result</th>
<th>Support for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tosh et al. (2007)</td>
<td>Confusion/oddity</td>
<td>Yes</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>White, Black cryptic</td>
<td>White</td>
<td>Prefer</td>
<td>Both</td>
<td>white preference</td>
</tr>
<tr>
<td>White, conspicuous White</td>
<td>White</td>
<td>No</td>
<td>Prefer</td>
<td>Both black</td>
</tr>
<tr>
<td>Black, conspicuous Black</td>
<td>Black</td>
<td>Yes</td>
<td>Prefer</td>
<td>Oddity black</td>
</tr>
</tbody>
</table>

689

690
Figure legends

**Fig. 1 a)** The proportion of attacks on cryptic *Daphnia* by three-spine sticklebacks in the three different prey ratio treatments (ratio of cryptic:conspicuous individuals for each treatment are as follows: 1:11 Cryptic minority, 6:6 Even, 11:1 Cryptic majority). Dotted lines indicate the expected proportion of attacks targeting a cryptic *Daphnia*, based on random expectation. Stars indicate significant differences between observed and expected proportions in binomial tests for 1:11 ($P = 0.535, N = 38$), 6:6 ($P < 0.001, N = 40$) and 11:1 ($P < 0.001, N = 38$). **b)** Per capita predation risk for cryptic (open circles, dashed line) and conspicuous (filled circles, solid line) individuals as the number of individuals of each type in the group increases.

**Fig. 2** The proportion of time spent by black and white colour morph mollies (test fish) with colour-matched shoals for conspicuous (grey bars) and cryptic individuals (open bars), mean ± 2 SE. Significant effect of fish colour (GLM, $t = 2.861, df = 106, P = 0.0083$) and fish crypsis ($t = -2.320, df = 105, P = 0.0366$) on the proportion of time spent with the colour-matched shoal was found. The horizontal dashed line indicates a random expectation of equal time spent with each shoal. Asterisks indicate significant ($P < 0.05$) deviation from this expectation based on one-sample t-tests (black-conspicuous: $df = 25, P < 0.001$; black-cryptic: $df = 25, P = 0.367$; white-conspicuous: $df = 28, P < 0.001$; white-cryptic: $df = 26, P < 0.001$).
Figure 1

Cryptic minority  Even  Cryptic majority

Proportion of attacks on cryptic Daphnia

Prey ratio treatment

Per capita predation risk

Number of colour–matched individuals
Figure 2

Proportion of time with colour-matched shoal

Test fish colour
Mixed phenotype grouping: the interaction between oddity and crypsis

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Table of key terms

The table below summarises the key terms that we use in the manuscript.

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confusion effect</td>
<td>A benefit of group living, whereby the attack-to-kill ratio of a predator (success rate) is reduced when individual prey animals aggregate (Miller 1922; Krakauer 1995; Krause &amp; Ruxton 2002).</td>
</tr>
<tr>
<td>Oddity effect</td>
<td>A mechanism where predators attack individuals within a group that are phenotypically distinct from the majority of the group (“odd”; Milinski 1977; Ohguchi 1978; Krause &amp; Ruxton 2002). The confusion effect and oddity effect operate together select for behaviours in prey leading to the evolution of phenotype-assorted groups (e.g. preference for associating with phenotypically matched group-mates).</td>
</tr>
<tr>
<td>Phenotype-assorted group</td>
<td>A group of individuals that are visually very similar. The terms “uniform group” and “homogeneous group” are equivalent (Krause &amp; Ruxton 2002)</td>
</tr>
</tbody>
</table>
### Mixed-phenotype group

A group of individuals that differ in appearance from one another (Tosh et al. 2007). This might include variation in appearance within the same species, or groups of two or more species (mixed-species group).

### Visual background

The background against which a prey animal would be viewed by a predator (Endler 1990; Ruxton et al. 2004). We distinguish here between two components of the visual background: the habitat (substrate/tank wall), and the other individuals in the group (for schooling fish, this may be an equally or more important component of the visual background than the habitat; Endler 1990). We use different terminology when discussing an animal’s appearance relative to these different components of the visual background.

### Conspicuous

An animal that stands out (visually) against the visual background (Ruxton et al. 2004). Here, we use ‘conspicuous’ or ‘cryptic’ to refer to the contrast/similarity between the animal’s body colouration and the colour of the habitat or substrate, and ‘odd’ or ‘phenotypically-matched’ to refer to the contrast/similarity between the animal’s colouration and the other members of the group.

### Cryptic

An animal that closely matches (visually) the characteristics of the habitat (Ruxton et al 2004).

### Odd

An individual which is phenotypically distinct (visually) from the other members of the group (Milinski 1977; Ohguchi 1978; Krause & Ruxton 2002)

### Phenotypically matched

An individual which is of the same visual phenotype as the other members of the group

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### References


