

Experimental evidence of specialised phenotypic roles in a mobbing raptor

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Abstract Group living is associated with costs but also with potential benefits, such as a decrease in predation risk through, for example, higher defence efficiency. Mobbing is among the most specialised forms of anti-predator strategies involving group defence and has mainly been investigated in passerine birds and some mammals. Variation in the mobbing response has been found in several species according to phenotypic variation such as sex or age. This suggests that there are differential benefits between mobbers, which may have promoted individual specialisation in mobbing behaviour. We studied mobbing behaviour in a communal roosting raptor, the Marsh harrier (*Circus aeruginosus*), which shows active group defence. Our study population exhibits extreme colour polymorphism, with two colour morphs in males, as well as sexual dichromatism and colour variation with age. We used different decoys, placed at different distances from the roost, to manipulate experimentally the perceived predation risk and to elicit mobbing behaviour. Using the experimental design that maximised mobbing response in harriers, we then focused on the sequence and the specific behaviours involved in recruitment of mobbers, and whether individual investment in terms of defence was associated with phenotypic characteristics of individuals (i.e. sex, age and colour morph). We found that the main behaviour involved in successfully attracting mobbers was alarm calling. We also detected differential individual investment in relation to sex and age, but more importantly, we provide the first evidence for specialised male phenotypic roles during

mobbing events, signalled by colour polymorphism: grey males tended not to be involved in mobbing and almost never behaved as recruiters or mobbers, while brown males behaved mainly as recruiter birds. These findings suggest that colour morph may signal the individual's anti-predatory abilities through different behavioural strategies between males.

Keywords Mobbing · Anti-predator strategy · Polymorphism · *Circus aeruginosus* · Raptor · Phenotypic specialisation

Introduction

Though in most cases prey tend to escape the attacks of predators (Lima and Dill 1990; Lima 2009), sometimes they do not: in particular, social species may be able to defend themselves against a predator, and such an ability has been considered to be a driving force for the evolution of group living and sociality (Alexander 1974; Wilson 1980; Hill and Dunbar 1998; Hass and Valenzuela 2002). Predation risk may be minimised in groups as a result of increased predator detection or by group defence (Ruxton et al. 2004; review in Caro 2005). The latter can vary from a simple warning to conspecifics when a predator is seen, to very complex alarm calls (Templeton et al. 2005; Griesser 2009) and highly specialised behaviours. Mobbing belongs to the latter category. This anti-predator strategy is widespread in birds, but has also been described in primates (Srivastava 1991), rodents (Owings and Coss 1977) and fish (Helfman 1989). Mobbing also occurs against social parasites such as cuckoos (Welbergen and Davies 2008). Mobbing usually consists of a suite of coordinated behaviours (i.e. a sequence). In songbirds, for instance, individuals first

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approach the predator and move rapidly around it (including inspection behaviour, close following, haranguing and even physical attack), perform wing and tail movements, and emit loud and broad-frequency calls (Curio 1978) that are recognised by conspecifics as well as heterospecifics (Marler 1957; Hurd 1996; Templeton et al. 2005).

Mobbing, however, incurs costs owing to prey injuries or death (Curio et al. 1983, 1985; Krams et al. 2007), it is time consuming (Collias and Collias 1978) and it brings energetic costs (Conover 1987). For mobbing to be adaptively maintained, the benefits from mobbing must outweigh these costs. The compensatory benefits of mobbing have thus formed the central focus of recent research (Caro 2005). The most straightforward benefit of mobbing relies on the ‘move-on hypothesis’, which states that a predator should leave an area sooner the more intensively and/or longer it is harassed. Under the move-on hypothesis, it is in the interests of each mobber to drive the predator away (Curio 1978; Woodland et al. 1980; Caro 2005). However, mobbing may also serve to advertise individual quality to other group members (Dugatkin and Godin 1992; Zahavi and Zahavi 1997; Maklakov 2002), to transfer information regarding the risk of threats, allowing prey individuals to respond adaptively (Dugatkin and Godin 1992; Brown 2003; Griesser 2009), or to teach individuals how to deal with predators (Graw and Manser 2007; see also Caro 2005 for a review). Whatever its function, the overall benefits of mobbing mainly depend on the attraction of conspecifics and heterospecifics to participate in defence by the process of recruitment (Curio 1978; Desrochers et al. 2002) because the recruitment of others allows an increase in mobbing efficiency toward the predator (Dugatkin and Godin 1992). The success in recruiting mobbers is thus a key to successful mobbing (Frankenberg 1981; Ostreiher 2003), though surprisingly, few studies have investigated mechanisms and specific behaviours eliciting recruitment.

Individual variations in mobbing response in relation to particular phenotypes, such as sex, age (Maklakov 2002; Graw and Manser 2007) or condition (e.g. Slagsvold 1985), have been found in several species, suggesting differential benefits to individuals during mobbing. Similarly, the number of recruited mobbers increases with alarm call rate (Arroyo et al. 2001), indicating individual variation in recruitment success. Anti-predator behaviour may thus vary according to individual phenotype, possibly leading to individual specialisation in mobbing response. A particular case of phenotypic variation, especially frequent in birds, is colour polymorphism. Though colour polymorphism and its associated function have been investigated repeatedly, mobbing in relation to coloration has seldom been studied (but see Negro et al. 2007), and individual response variation in mobbing in relation to plumage coloration has

never been investigated. This is surprising since colour polymorphism is known to co-vary with morphology, physiology and behaviour (see Roulin 2004 for a review). In particular, melanin-based coloration is associated with aggressiveness, a relation that results from pleiotropic effects of the genes regulating the synthesis of the pigment (Ducrest et al. 2008). Morgan et al. (2004) found that melanocortins promote aggressiveness by inducing the production of aggression self-stimulating pheromones through binding to a particular receptor, and Ducrest et al. (2008) found that dark eumelanic individuals were more aggressive than lighter ones from the same population in 18 out of 20 species for which data were available. Colour polymorphism may therefore be associated with aggressiveness and thus affect individual investment in mobbing.

Here, using decoys to manipulate predation risk experimentally, we elicited mobbing behaviour in a gregarious, polymorphic, medium-sized raptor, the Marsh harrier *Circus aeruginosus*. This species, like several other harriers, roosts communally on the ground and is thus particularly vulnerable to predation (Cramp and Simmons 1980). Harriers show active anti-predator defence, i.e. predator detection, alarm calling, recruitment of congeners (with up to 50 mobbers; personal observation) and active attack of the predator (Simmons 2000; Arroyo et al. 2001). In addition, this particular species has evolved strong sexual dichromatism as well as plumage colour polymorphism. The latter is presumably melanin-based, as in other raptor species (Ferguson-Lees and Christie 2001; Fargallo et al. 2007). In our study population, two male colour morphs co-exist, with males being either ‘grey’ or ‘brown’ (Bavoux et al. 1993, 2006), depending probably on relative concentrations of eumelanin and pheomelanin in their feathers (see Haase et al. 1992; Wolff 2003). In addition, individuals of both sexes show extreme variation in general coloration, between dark and light, but also becoming lighter with age. We first experimentally assessed whether exposure to a decoy elicited a mobbing response from the harriers and whether this response varied in relation to the type of decoy and its distance from the roost. Then, having identified the combination of decoy type and distance that provoked the strongest mobbing response, we (1) analysed the sequence of mobbing behaviour and identified specific behaviours that elicit recruitment, and (2) assessed whether mobbing behaviour depends on individual phenotype. To analyse mobbing sequences in detail, we defined and quantified mobbing behaviour and its effect in recruiting congeners. We predicted that alarm calls, such as in the majority of species exhibiting mobbing, should elicit recruitment and that efficiency in recruitment should correlate with alarm call rate. In addition, because we were able to sex and to some extent age birds, and to classify males according to their colour morph, we analysed individual mobbing

behaviour in relation to phenotype in order to demonstrate differential investment in mobbing. Furthermore, if melanin-based coloration is associated with behavioural traits (Ducrest et al. 2008), we expected males of different colour morphs to behave differentially during a mobbing event.

Materials and methods

Study area and species

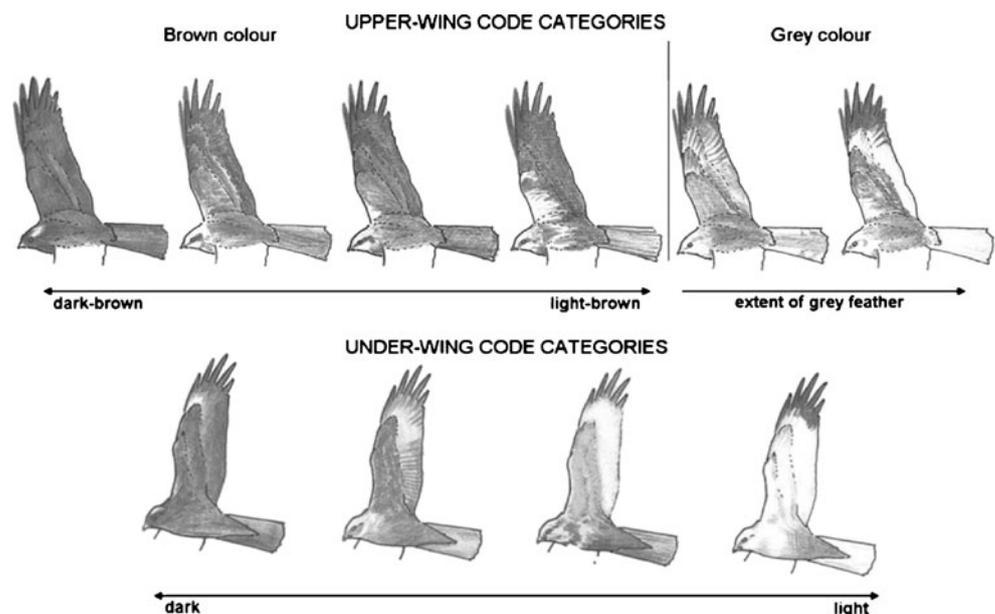
Fieldwork was conducted during three winter seasons (2005–2006, 2006–2007 and 2007–2008) on a sedentary population of Marsh harrier in the Marais de Brouage (central western France, c. 120 km², 45°51'N–01°04'W; see Sternalski et al. 2008 for additional details). The study site was mainly composed of grassland (86%) and cultivated area (14%), with numerous small reed beds that are used by Marsh harriers for communal roosts (Bavoux et al. 1997). All night roosts within the study area were searched and located in the evening. Communal roosts differed with regard to vegetation type and accessibility by car. Each year, we chose three to four roosts of varying size out of ca. 25 available roosts to perform the tests; in total, tests were carried out on seven different roosts. These roosts were surveyed every week during the season, allowing us to estimate the size of each roost used in the tests. The average roost size was 23.4 harriers (range 1–85).

General coloration of flying birds was assessed with colour scores (for a view of different colour morphs, see Bavoux et al. 1991, 1993 and Forsman 2008 for some photographs; see also Fig. 1). Plumage scores were obtained by assigning a code category to the upper-wing

and under-wing of each bird in the field. Four colour categories for under-wing (ranging from very dark to light) and six colour categories for upper-wing (four codes for brown colour, from completely dark brown to light brown with very large white shoulders, and two codes for grey colour, according to the extent of grey feathers in the plumage) were available on a chart used by observers in the field (Fig. 1). The two male colour morphs were easily categorised by the presence or absence of grey feathers in the upper-wing, and were thus split as 'grey' and 'brown' (i.e. female-like) morphs, respectively. During the winter of 2006–2007, we estimated plumage coloration of the entire population by performing low-speed (20 km/h) and standardised car transects within the study site ($N=25$ transects). For each transect, two observers sexed and categorised plumage coloration of each observed individual harrier flying or landed within 500 m from the car. A total of 512 individuals was thus scored (we cannot exclude the possibility that the same individual was scored several times), which provided a population distribution of colour scores. Grey males accounted for nearly two thirds of the entire male population, based on these observations.

Females and brown males look alike in this population, and therefore sexing birds in the field was quite difficult if based only on coloration (Bavoux et al. 2006), but feasible using bird silhouettes. During the three winter seasons, a total of 271 counts were conducted on 25 different roosts within the study site (188 counts available from the seven roosts used in tests). We thus sexed 1,498 birds visually over the course of this study (i.e. including birds sexed during tests and also during roost counts) and found a population sex ratio of 50.8% male, close to the population sex ratio previously obtained from captured birds (49.1%,

Fig. 1 Colour variation in flying Marsh harriers



$N=648$; Bavoux et al. 2006) and not statistically different ($\chi^2=0.58$, $df=1$, $P=0.45$). A high proportion of birds (22%), however, could not be sexed with certainty in the field: sex ratio analyses were based only on sexed birds.

The age of individuals could be assessed for two categories of bird. First, fledglings of the previous year (i.e. one calendar-year-old birds) are easy to identify because of their new plumage. Secondly, nearly all fledglings were individually wing-tagged in 2006, which allowed ascertaining the age of a small cohort (less than 10%) of birds. Overall, we were thus able to record sex, age and plumage coloration of individuals.

Experimental design

Decoy presentation tests

We first assessed whether Marsh harriers responded to decoys as predators and whether one experimental design in particular elicited a stronger mobbing response. Over the course of the study, 122 tests were performed, each using one of three different decoys: stuffed fox, plastic eagle owl and plastic crow. We could not find a plastic fox and thus used a stuffed specimen (see Arroyo et al. 2001; Krams et al. 2008 for similar procedures), but preferred to use manufactured objects when these were available. Foxes and crows were common in the study area, whereas the eagle owl (hereafter owl) was absent. The fox and the owl, being ground and avian predators, respectively, represented potentially a direct risk for harriers roosting on the ground, despite owls being absent from the study area and consequently unknown to this sedentary bird population. In contrast, the crow was not dangerous to adult roosting birds but may have been identified by harriers as an egg predator. In addition, we also manipulated the distance between the decoy and the roost. Therefore, for each test, one of the three randomly chosen decoys was placed either 'adjacent' to the roost or 'far' from it (50–100 m). Decoys were always placed before birds arrived at the roost 30–60 min before sunset. The fox was placed on the ground, and the owl and crow on a 1-m-high perch. Tests were performed under favourable weather conditions and were stopped as soon as light conditions were not sufficient to score the colour of birds.

Once the decoy was settled, the observer went back to the car and started to observe at a distance of 50–200 m. A test started as soon as a harrier flew within 100 m of the decoy and detected the decoy by exhibiting a conspicuous behaviour such as turning its head toward the decoy while flying in another direction or modifying its trajectory and flying toward it. Tests were intended to last 15 min from the starting time, but due to the fieldwork constraints, the exact duration of tests was on average 14 ± 3 min. During this

time, we recorded every bird coming to the area, its age, sex, plumage characteristics and behaviours (see below for categories). Birds were colour-scored, as far as possible, when they arrived in the proximity of the roost and before they performed any behaviour, to limit observer bias. After the 15 min had elapsed, irrespective of outcome (i.e. recruitment occurred or not), we removed the decoy. Finally, the trial was stopped and the test was declared 'negative', and thus not considered for analyses if, within 30 min after decoy settlement, no harrier has been observed within 100 m of the decoy.

Bird categories and behaviour definitions

We distinguished between detection and mobbing behaviours. Detection behaviours that were counted and noted were (a) simple flight over the decoy within 20 m of it (Fly-over) and (b) conspicuous climb of 2–3 m and hovering flight just above the decoy (Hovering), which we interpreted as the bird being surprised (by the decoy). Mobbing behaviours that were counted and noted were (c) specific circling flight over the decoy (Circles), (d) alarm calling (Alarm) and (e) repeated dives over the decoy (Dive). These latter behaviours are those typically identified as mobbing behaviour in several bird species (Curio 1978).

Because of extreme inter-individual variation in plumage coloration and the reduced number of birds involved in the first stages of tests (i.e. before any recruitment occurred), all individuals before a recruitment event could be monitored individually. Once recruitment occurred, however, it was not possible to monitor accurately all the individuals involved in the mob, and thus only the number of mobbers (group size) was noted as well as the behaviour of the focal individual that recruited the mobbers. A typical mobbing recruitment sequence would include a harrier first detecting the decoy, then a harrier, being the same individual or not, performing one or several mobbing behaviours, and then a group of recruited mobbers if recruitment occurred. We classified individual Marsh harriers, a posteriori, into three individual categories on the basis of the time sequence and nature of behaviours they performed. The first harrier that detected the decoy could either only showed detection behaviours, such as fly over or hovering above the decoy, and thus remained strictly a DETECTOR, or showed mobbing behaviours and thus became a RECRUITER, being the same as the first bird approaching the decoy, or not. Finally, the MOBBERS were all birds that joined the mob party, which could thus include the recruiter bird itself and/or the detector bird. We never recorded detectors that were joined by mobbers without performing mobbing behaviour. A test started therefore with the detection of the decoy by a detector. We then considered that a decoy elicited an attempt at recruitment when a mobbing

behaviour (circle, alarm or dive) was performed by a harrier, and that recruitment occurred when a harrier being a recruiter was joined by at least one other (i.e. a mobber).

Statistical analyses

A grand total of 122 experimental tests were performed within the 15-min test period, but in order to increase sample size, we extended the duration of 17 negative tests in 2008. Recruitment occurred in eight of these 17 tests (providing a total of $N=41$ tests ended with recruitment). These 17 tests were however considered only for the analysis of behaviours during the mobbing sequence. In addition, during a same trial when the first harrier exhibited mobbing behaviour but failed to recruit mobbers, a second (and possibly a third) bird generally came within the 15-min test period (and therefore, the mobbing recruitment sequence was repeated). We also used these additional sequences to increase the sample size. We checked that this did not introduce bias: using the 35 trials performed in 2008 with the fox adjacent to the roost, 34 trials led to decoy detection, but in 15 of these (44.1%) no recruitment occurred. In such cases, the first bird left the decoy, and in 13 cases (86.7%) a second harrier (always different) came over the decoy on average 11 ± 11 min (range 1–39) later. Again repeating the same analysis, 66.7% of these secondary birds did not recruit other harriers, and in 83.3% of such cases a third harrier came and detected the decoy. We found no significant differences between sequences (first to third) in detection behaviour frequency (Fisher exact test— $F=0.06$, $P=0.18$, $N=57$) and mobbing behaviour frequency (Fisher exact test— $F=0.05$, $P=0.32$, $N=53$). As we were interested in the succession of behaviours that led to recruitment, we therefore pooled all data for the analyses, irrespectively of whether the individual harrier was the first, second or third, providing a total of 396 individual birds for which behaviour was quantified. Pseudoreplication might thus be an issue in our analyses, since the same roosts were used several times and the same individual birds may have been involved an unknown number of times (as they were not individually tagged). Pseudoreplication may thus bias our results, either in inflating the statistical power of tests or possibly also in over-representing specific categories of individuals. In order to account for pseudoreplication, we used generalized linear mixed models (GLMM) using PROC GLIMMIX (SAS 2001). When the basic statistical unit was the test, roost identity where the test was performed was included as a random effect. When the basic statistical unit was the individual harrier, test identity was further included as a random effect. We have no way to account for the fact that the same harrier may be involved repeatedly across different tests in different days (although we were able to

separate individuals within tests), and our results using individuals must therefore be interpreted with caution. According to the distribution of errors, we either used normal or binomial models (mixed and logistic regressions) to estimate the effects of different factors or of their interactions on the probability of decoy detection, attempted recruitment and successful recruitment (the independent variables).

Behaviour data were analysed as presence–absence data (1 indicated that a given individual performed a given behaviour, irrespectively of how many such behaviours were performed) or as the number of behaviours per minute. In order to analyse which mobbing behaviour elicited recruitment, we distinguished between individuals observed before any recruitment occurred ($N=267$ excluding both mobbers and recruiter birds once recruitment started) and those after recruitment ($N=129$, including 104 mobbers and 25 recruiter birds once recruitment started). In order to analyse coloration of individuals, we performed a multiple correspondence analysis (MCA) on colour codes to obtain a single and linear index of coloration of each harrier. MCA is a simple correspondence analysis carried out on an indicator matrix with individual harriers as rows and categories of colour codes as columns, and can be seen as an equivalent of principal component analysis for discrete (or qualitative) variables in that it allows reduction of the number of original variables (the colour codes) to a lower number of orthogonal axes. We retained the first axis of the MCA, which accounted for 64.2% of the total variance, and used the coordinate of each individual (irrespectively of its sex or age) on the first axis as an index of overall coloration. MCA was run on the colour scores obtained from the 512 individuals scored during car transects as well as individuals involved in mobbing sequences.

Non-parametric tests were used for frequency data; means are given \pm SD, and all statistical tests were performed with SAS v.9.

Results

Effect of decoy and distance on mobbing behaviour

The proportion of negative tests was 12.3% ($N=15$ among the 122 trials performed). Overall we found no effect of start time of test (relative to sunset) or year on the probability of either decoy detection or recruitment (GLMM, with roost identity as random effect; all P values being >0.14), but we found a slight year effect on the probability of an attempt to recruit (i.e. at least one mobbing behaviour shown by a harrier; $F_{2,105}=3.87$, $P=0.02$, $N=122$). However, this effect was mainly due to decoy type because entering this variable in the model removed the year effect (year— $F_{2,98.8}=2.48$, $P=0.09$; decoy type— $F_{2,98.8}=2.30$, $P=0.11$; interaction—

$F_{2,98.8}=2.38$, $P=0.01$). Therefore, we pooled all tests for subsequent analyses examining the effects of decoy type, decoy distance from the roost (either adjacent or far from roost) and roost size.

The proportion of trials with decoy detection was positively affected by roost size, as would be expected simply because more birds were moving around (Table 1). Attempted recruitment was significantly affected by decoy type, with fox eliciting more mobbing behaviours than owl and crow, respectively (Table 1, Fig. 2). Most successful recruitment events were in response to the fox, whatever the distance and the roost size or, to a lesser extent, to the owl adjacent to the roost (Table 1, Fig. 2). Considering only experimental trials with decoy detection (resulting in 107 trials), 70 (65.4%) involved a bird attempting to recruit, and 33 (30.8%) ended with successful recruitment of conspecifics. The mob size (excluding the recruiter) was highly variable (6.56 ± 7.5 individuals; range 1–26), though none of the variation was accounted for by decoy type (fox and owl elicited on average 7.6 ± 7.9 , $N=28$ and 2.2 ± 1.3 harriers, $N=5$, respectively; Mann–Whitney, $Z=-1.45$, $P=0.15$) or roost size (log-transformed values; $F_{1,32}=0.84$; $P=0.37$). Therefore, harriers responded differentially in relation to experimental design; the fox decoy settled adjacent to the roost eliciting the strongest mobbing behaviour and recruitment. This design was thus used in winter 2007–2008 to maximise the number of sequences of events leading to recruitment.

Behaviour of detectors, recruiters and mobbers

Using the 267 individual harriers for which behavioural data were collected before any recruitment event occurred, we found that only alarm calls and, to a much lesser extent, circle flights significantly and positively elicited recruitment, while fly-over significantly and negatively affected the probability of recruitment (Table 2). We used this result to categorise arbitrarily the 267 individual harriers, splitting

individuals between detectors (showing only detection behaviours, i.e. fly-over and hovering—38.6% of birds) and recruiters (showing at least one mobbing behaviour; 61.4%). We further split recruiters between those that succeeded in recruiting mobbers (labelled as successful recruiter, 17.6%) and those that failed (failed recruiter, 42.7%). These proportions differed significantly with decoy type ($\chi^2=34.6$, $df=4$, $P<0.0001$, $N=267$) and decoy distance from the roost ($\chi^2=11.8$, $df=2$, $P=0.003$). For instance, the proportion of successful recruiters was null in front of the crow decoy, and increased from owl to fox (from 10% to 23% for owl and fox, respectively), being always higher when decoy was adjacent to the roost than far from it (from 0% to 12% and 13% to 25% for far and adjacent to the roost, respectively, for owl and fox).

Among recruiters, we compared failed and successful individuals, and found that the only behaviour that significantly differed between the two categories in predicting recruitment success was alarm calling (multiple stepwise logistic regression— $\chi^2=26.2$, $df=5$, $P<0.0001$, $N=161$). Recruiters that failed gave an alarm call in only 14.5% of cases while 55.3% of successful recruiters alarm called. The number of alarm calls given per minute also showed a marked difference: 0.35 ± 1.28 vs 1.55 ± 2.6 alarm calls, respectively ($F_{1,91}=12.2$, $P=0.0007$). Once (and if) recruitment occurred, we compared the behaviour of the former recruiter bird and that of the mobbers ($N=129$ individual harriers). We found that mobbers gave significantly fewer circle flights and alarm calls than former recruiter birds (multiple stepwise logistic regression, dives excluded from the model— $\chi^2=22.1$, $df=4$, $P=0.0002$). In addition, former recruiter birds never dived, presumably the most risky behaviour during mobbing, which suggested that, after recruitment, former recruiters still behaved as recruiters rather than mobbers. This was actually confirmed by the fact that their behaviour did not significantly differ between the two periods (i.e. before and after recruitment

Table 1 Effects of decoy type, distance between decoy and roost (i.e. either adjacent or far to the roost), and roost size (log-transformed) on probability of decoy detection, attempted recruitment (i.e. at least one mobbing behaviour showed by a harrier) and successful recruitment

Source of variation	Probability of decoy detection			Probability of attempt to recruitment			Probability of recruitment event		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
<i>N</i>	122			107			70		
Decoy type	2, 114	1.82	0.17	2, 99.6	3.84	0.02	–	–	–
Distance	1, 90.9	2.68	0.10	1, 100	3.47	0.06	1, 65.9	0.10	0.75
Roost size	1, 66.7	6.18	0.01	1, 96.6	3.63	0.06	1, 65.4	0.27	0.60
Size*Distance	1, 95.4	0.25	0.62	1, 99.6	0.02	0.89	1, 65.7	0.62	0.43

Mixed logistic models including roost identity as a random effect, were used. Some terms were sometimes omitted because the model did not converge

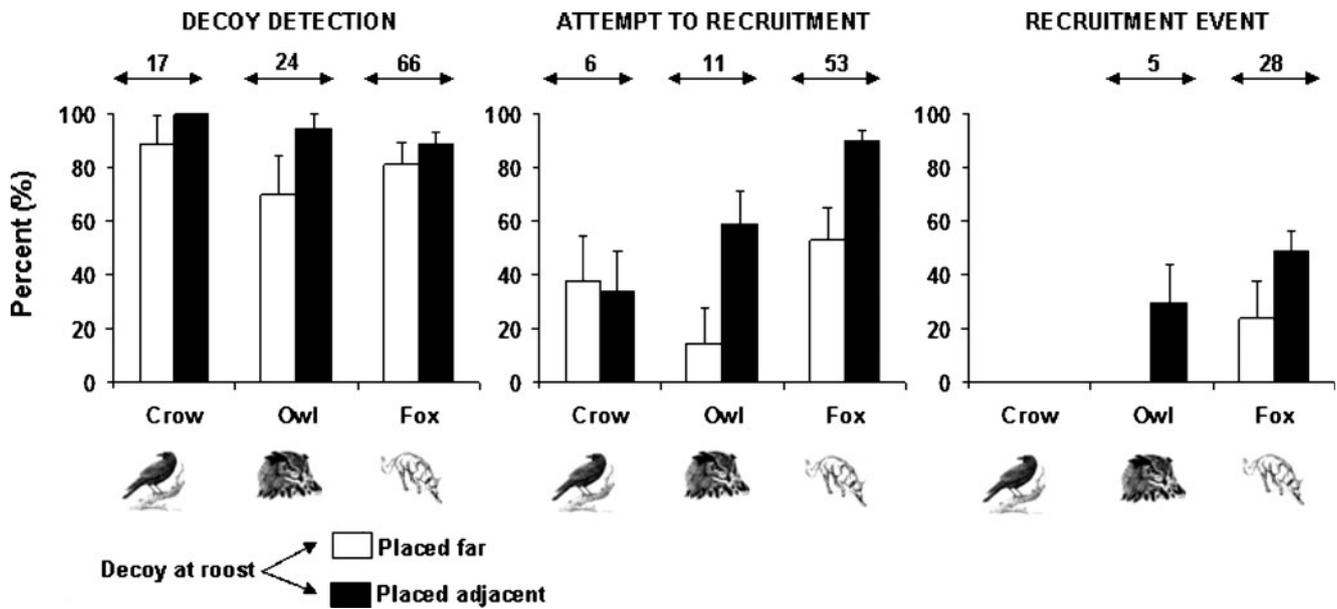


Fig. 2 Results of the decoy presentation tests. Effects of decoy type and distance from roost on (left–right) probability of decoy detection, attempted recruitment (i.e. at least one mobbing behaviour showed by

a harrier; see ‘Materials and methods’) and successful recruitment. Number of successes are provided within brackets. Error bars are standard errors of the binomial distribution

occurred; multiple stepwise logistic regression, dives excluded from the model— $\chi^2=7.2$, $df=4$, $P=0.13$, $N=69$).

Phenotypic specialisation during mobbing

There were no apparent differences in sex ratio between the five categories of birds (Fig. 3): detectors, failed and successful recruiters, mobbers and former recruiters during the mob (mixed logistic model with the probability of being male-modelled, test identity as random effect— $F_{5,297}=0.55$, $P=0.74$, $N=310$ birds that could be sexed out of the 396), even when restricting the analysis to the time before recruitment occurred ($F_{3,201}=1.02$, $P=0.38$, $N=205$). On average males accounted for 59% of birds involved (Fig. 3). This contrasted with the overall sex ratio at roosts (as a proxy of sex ratio of the wintering population), which was 51% ($N=$

1,498 sexed birds), a difference that was significant ($\chi^2=6.86$, $df=1$, $P=0.008$). Therefore, it appeared that males were significantly over-represented in the sequence of mobbing (an excess of ca. 10%) compared to the roosting population. With regard to age, we never detected a yearling (one calendar-year-old bird) in any mobbing event, and 2-year-old wing-tagged birds only accounted for 4.3% of all birds involved, and never behaved as recruiters. Overall, therefore, harriers involved in mobbing were mainly adult birds.

Plumage was analysed through the plumage scores obtained by MCA (see Fig. 4a for the distribution of individual colour scores in the male wintering population obtained by MCA). It clearly revealed that males that were involved in the mob sequence ($N=110$) strongly differed in their overall coloration in comparison to males at the roost ($N=512$; Kolmogorov–Smirnov test, $D=0.6$, $P=0.009$;

Table 2 Number and proportion of individual harriers showing each of the five recorded behaviours (see ‘Materials and methods’ for definitions) before any recruitment event occurred

Behaviour	Before recruitment (%) <i>N</i> =267 individuals	Effect on probability of recruitment			
		Estimate (SE)	<i>df</i>	<i>F</i>	<i>P</i>
Fly-over	154 (57.7)	-1.19 (0.33)	1,265	13.2	0.0003
Hovering	59 (22.1)	-0.08 (0.37)	1,264	0.05	0.82
Circle	145 (54.3)	1.04 (0.34)	1,265	9.4	0.03
Alarm	43 (16.1)	2.65 (0.37)	1,203	51.5	<.0001
Dive	16 (6.0)	0.01 (0.62)	1,246	0.001	0.98

The effects of these behaviours on a recruitment event were tested using a mixed logistic model (including test identity as a random effect)

Fig. 3 Sex ratio of harriers involved in mobbing sequences, according to their behavioural category before (*left*) and after (*right*) recruitment (see text for details). The *dotted line* represents the average sex ratio for the whole population, as established from counts at roosts when no test was carried out (see ‘Materials and methods’)

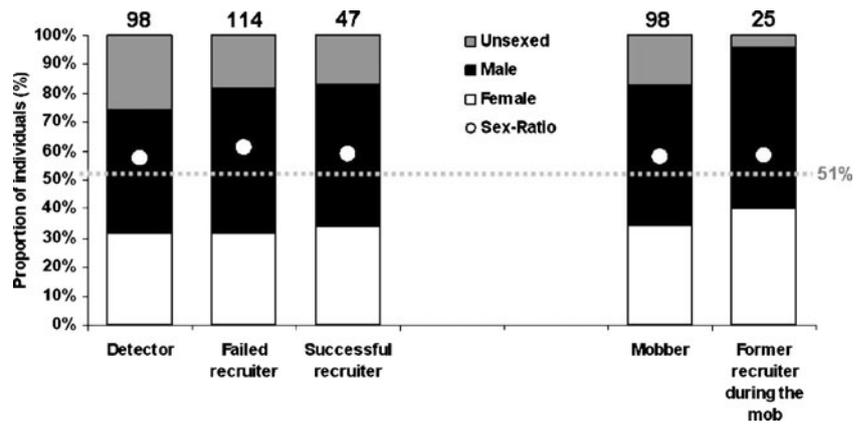


Fig. 4b). Colour score was actually a significant predictor of male category (multinomial GLM; $\chi^2=4.36$, $df=1$, $P=0.03$; $N=110$ males). In summary, grey males were significantly less involved in mobbing than their availability in the roost population, though in the case of male detectors, they were equally brown (negative plumage scores) or grey (positive plumage scores; Fig. 4c). Accordingly, we found that brown and grey males performed behaviours differentially: grey males exhibited fewer alarm calls (mixed model with test identity as random effect— $F_{12,58}=2.28$, $P=0.02$, $N=107$) but more circle flights ($F_{12,58}=2.56$, $P=0.009$) than brown males. In contrast to grey males, the distribution of colour scores in brown males did not differ between the whole roosting population and the individuals involved in mobbing ($D=0.5$, $P=0.16$, Fig. 4). However, failed and successful brown male recruiters differed in their coloration: failed recruiters were significantly darker than successful recruiters (Kruskal–Wallis test, $\chi^2=5.1$, $df=1$, $P=0.02$, $N=42$; Fig. 4d–e). There was no such difference in female plumage colour score distribution between the roost population and those involved in mobbing, although, as in male, an apparent lack of young birds (dark) involved in mobbing events (data not shown; $D=0.466$, $P=0.07$).

Discussion

In this experimental study, we first investigated the mobbing response of Marsh harriers by manipulating the decoy type and its distance from the roost site. We found that harriers responded less to a (presumably) non-dangerous predator (i.e. the crow) than to a more dangerous predator (the fox), but also responded more when the decoy was close to the roost. Functional effects of mobbing (i.e. predator deterrence, predator risk assessment and transfer of information) all predict that mobbing intensity will be correlated with threat level (Helfman 1989; Dugatkin and Godin 1992; Brown 2003). Harriers were apparently able to identify decoys as predators and associate predator recog-

nition with level of threat (Altmann 1956), as suggested by their modulation of group defence toward different decoy types. Other studies involving mammals, e.g. vervet monkey (*Cercopithecus pygerythrus*, Seyfarth et al. 1980), but also birds, e.g. Siberian jays (*Perisoreus infaustus*, Griesser 2009), have demonstrated that prey species may signal predator dangerousness by producing acoustically distinct alarm calls and respond with behaviourally adaptive escape responses. We must remain cautious however because, in our study, variation in mobbing response may result partly from an artefact, i.e. a difference between stuffed and plastic decoys. In addition, what prey perceive as risk depends highly on the behaviour and the state of predators (e.g. see Griesser 2008), and it is sometimes difficult to assess the risk that is posed by different predators based solely on the reaction of prey because some prey actually show reduced mobbing response towards more dangerous predators (Templeton et al. 2005; Griesser and Ekman 2005). Further investigations are therefore needed to assess whether Marsh harrier mobbing response is adaptive in relation to risk assessment.

Our next aim was to analyse the sequence of mobbing behaviours, and the particular displays, that elicited recruitment. Mobbing behaviour is usually mediated through the use of alarm calls (Bradbury and Vehrencamp 1998), though some species do not emit them during mobbing (Jones and Hill 2001). These are designed to maximise transmission to the predator and/or conspecifics, facilitate group formation and cohesion, and possibly to manipulate the behaviour of conspecifics (Charnov and Krebs 1975). Although many studies have investigated the relationship between alarm calling and mobbing (reviewed in Ellis 2008), few have accurately assessed the sequence of mobbing behaviours inducing recruitment. Our results indicated that alarm calling, and to a lesser extent circle flight, significantly elicited recruitment. Harriers used, therefore, both acoustics and visual signals to attract conspecifics, but acoustic cues were apparently more efficient. In addition, some visual cues other than displays

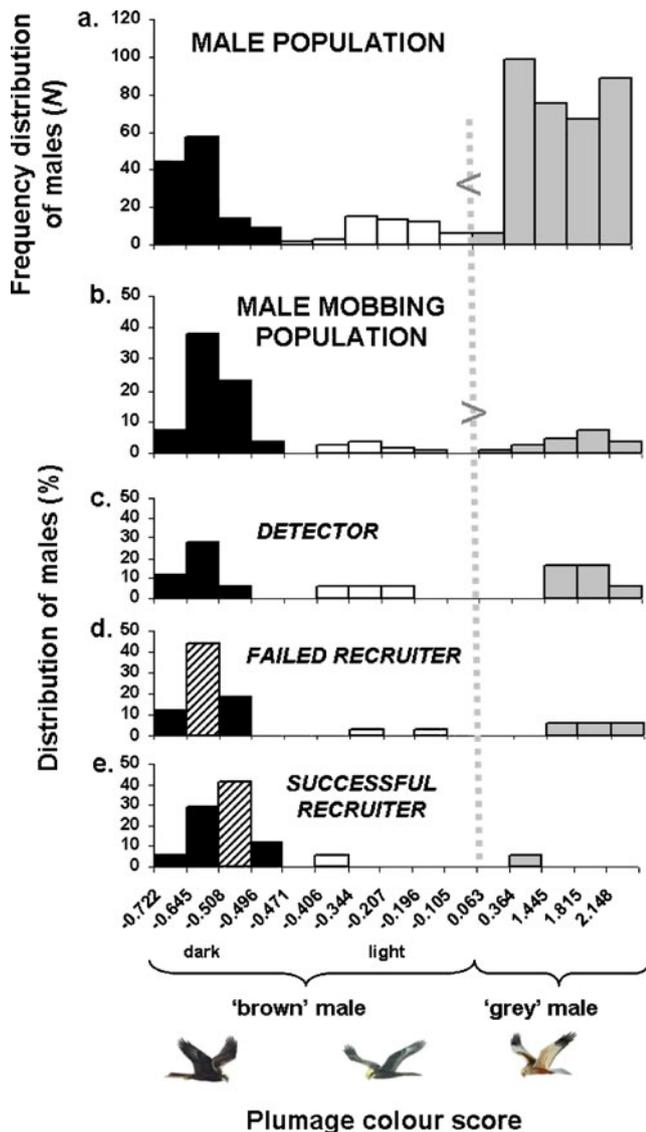


Fig. 4 Frequency distribution of plumage colour score (coordinate on first axis of MCA; see ‘Materials and methods’) among male Marsh harriers **a** in the entire roosting population ($N=512$ birds); **b** involved at any stage of the mobbing sequence ($N=110$); and behaving as **c** detectors ($N=18$), **d** failed recruiters ($N=32$) or **e** successful recruiters ($N=17$). Coloration score was obtained by colour coding of the upper- and under-wing (see text), from extreme dark brown males (left) to light brown males (centre) and grey males (right). The dotted line separates the two male colour morphs (i.e. ‘brown’ and ‘grey’) for clarity

may have played a role, as the colour morph of the recruiter affected the probability of recruitment (see below).

We further tested whether birds of different sex or age invested differently in mobbing. Individual variation in mobbing response has been found to correlate with individual characteristics of mobbers (Hasson 1991). For instance, Knight and Temple (1988) found that red-winged blackbird females gave more ‘checks’ than males, whereas the latter approached the decoy more closely, and hovered over and stroked the decoy more than females. Conversely,

in meerkats (*Suricata suricatta*), males mob longer than females, and adults between 1 and 2 years of age mob longer and more intensively than adults over 2 years or young animals under 1 year of age (Graw and Manser 2007). They proposed that mobbing behaviour in meerkats might reflect sex differences in cooperative behaviours and might allow young to learn about predators by observing adults. In contrast, Maklakov (2002) showed that, in the Arabian babbler (*Turdoides squamiceps*), subordinate females mobbed longer than subordinate males and that immature birds mobbed longer than any other bird class. He suggested mobbing as self-advertisement for the formation of dispersal coalitions for subordinate females and mobbing as a means of learning about different types of predators for immature birds. In our study, we found that younger birds were much less likely to be involved in mobbing in proportion to their relative frequency. It is possible that young Marsh harriers may have to learn to recognise and categorise predator types before becoming an efficient ‘detector’, such as in vervet monkeys (Seyfarth et al. 1980). We also found that males were significantly more likely to participate in the mobbing sequence than females, in accordance with the individual quality advertisement hypothesis (Zahavi and Zahavi 1997; Maklakov 2002). Sex differences in mobbing behaviour have already been interpreted as evidence for self-advertisement (Maklakov 2002), and our study population being sedentary, males and females which mix in roosts are all potential mates for each other. Males may thus use communal roosts as a way to signal their attractiveness to potential mates. Indeed, risks taken by individuals during mobbing could potentially have a signalling function (Curio et al. 1983; Francis et al. 1989; Moholt and Trost 1989), such as ‘social prestige’ in cooperative species (Zahavi 1995; but see Wright 1997).

We lastly investigated whether plumage coloration could affect mobbing behaviour. Differently coloured birds behave in a different way, as has been shown in the case of melanin-based pigments, demonstrating a causal link between colour and behaviour (see Roulin 2004 for a review). In the Marsh harrier, grey and brown males presumably differ in their relative concentrations of eumelanin and pheomelanin pigments in their feathers: higher concentrations of pheomelanin produce reddish-brown and brown coloration, while higher concentrations of eumelanin result in grey and black coloration (Haase et al. 1992; Jawor and Breitwisch 2003; McGraw et al. 2005). Accordingly, we indeed found in our study that grey and brown males differed behaviourally during mobbing: grey males in the population were under-represented in mobbing sequences, were predominantly detectors (i.e. the first individuals to come), but were almost never recruiters or mobbers. Conversely, brown males were more present in mobbing events and behaved mainly as recruiter birds. We

also found that brown male efficiency in recruiting other harriers was affected by their coloration, with lighter males being more efficient than the darker ones, possibly in relation to age (in this species, older birds tend to be lighter). These findings provide evidence for specialised phenotypic roles during mobbing and, to a larger extent, highlight the potential existence of two different male behavioural strategies in our study population, signalled by their coloration morphs. Because grey males are likely to circulate more eumelanin than brown males and since high eumelanin concentration results in more aggressiveness, we suggest that grey males exhibit a ‘solitary’ strategy, whereas brown males exhibit a more ‘social’ strategy. Grey males would therefore avoid social interactions in winter (as for instance at roosts). In contrast, brown males would show a rather more social and cooperative strategy in winter. We might expect brown males in summer to adopt a less aggressive behaviour for breeding than grey males, such as a sneaky behaviour which was already shown in the ruff (*Philomachus pugnax*) in which dark males were aggressive and white males adopted a sneaky behaviour to copulate with females (Hill 1991; Lank et al. 1995; see also Tuttle 2003 for the white-throated sparrow *Zonotrichia albicollis*). In our case, however, further investigations concerning reproductive behaviour, and aggressiveness in relation to colour morph in particular, are warranted to confirm the existence of different behavioural strategies between colour morphs. At this stage, we have nevertheless provided evidence for specialised phenotypic roles during mobbing events in the Marsh harrier with regard to colour morph, which, to our knowledge, is the first such evidence available in group defence behaviour.

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