CSIRO PUBLISHING

www.publish.csiro.au/journals/emu

Emu, 2004, **104**, 267–271

A study of the species-confidence hypothesis with Spiny-cheeked Honeyeaters (*Acanthagenys rufogularis*)

Molly L. Gould[A], *Lauri Green*[A], *Brie Altenau*[A] *and Daniel T. Blumstein*[A,B]

[A]Department of Organismic Biology, Ecology, and Evolution,
University of California, Los Angeles, CA 90095-1606, USA.
[B]Corresponding author. Email: marmots@ucla.edu

Abstract. The species-confidence hypothesis states that birds are attracted to colours found on their own body and are repelled by colours not found on their body. Previous studies have examined this hypothesis by measuring the distance at which birds took flight in response to humans approaching them while wearing different-coloured clothes. One study found that birds without red or orange on their bodies had longer flight-initiation distances when an orange vest was worn, while birds with those colours on their bodies were not affected. We evaluated the species-confidence hypothesis by approaching Spiny-cheeked Honeyeaters (*Acanthagenys rufogularis*) while wearing four different-coloured T-shirts and noted their flight-initiation distance. We predicted that birds would allow us to approach closer if we were wearing a colour found on their body. Unlike previous studies, we accounted for the fact that different colours are differentially detectable in the field because detectability alone may influence flight-initiation distance (i.e. bright colours are more detectable simply because they are bright). Two shirts were highly detectable (red and yellow) and two were relatively cryptic (tan and olive). Specifically, Spiny-cheeked Honeyeaters were found to have the longest flight-initiation distances when approached by a person wearing a yellow T-shirt (a colour not found on the bird), and the shortest flight-initiation distances when approached by a person wearing a red T-shirt (a colour found on the bird). The species-confidence hypothesis may account for variable flight-initiation distances.

Introduction

The species-confidence hypothesis states that birds should have a preference for colours that appear on their own bodies over colours that do not appear on their body (Burley 1986). This may have evolved so that an animal could recognise another member of its species as a potential mate. In this way, colours that are not found on an animal's body are 'unattractive' because it makes them appear different from other members of its species, and therefore not a potential mate (Burley 1986). Female Zebra Finches (*Peophila guttata*) preferred to mate with red-banded males who had orange-red beaks, even over those without leg bands, and stayed away from males with light-blue and light-green bands, colours that do not naturally appear on finches (Burley *et al*. 1982). In a later study, Double-barred Finches (*Poephila bichenovii)* preferred blue-banded mates (they have bluish beaks) over unbanded and red-banded birds. Also, zebra finches preferred red-banded mates to unbanded and blue-banded birds (Burley 1986).

It is clear that birds can react to colours in the visible spectra. For instance, Weatherhead *et al*. (1991) showed that male Red-winged Blackbirds (*Agelaius phoerniceus*) were less likely to lose their territories if they had a large number of red bands on their leg that accentuated the indicator red epaulets on their wings. A later study demonstrated that the increase in territorial aggression was specifically associated with the red leg bands as opposed to the contrast between leg band colour and the colour of the birds' legs (Metz and Weatherhead 1993). Yellow markers placed on the heads of female Mourning Doves (*Zenaidura macroura*) caused the disruption of recently formed pair bonds. The yellow markers also prevented the reformation of these pair bonds when the females were reintroduced to the males after being separated for a few days (Goforth and Baskett 1965). In addition, experiments have used colours to evoke differential responses in a variety of other species (Pepperberg 1987; Slobodchikoff *et al*. 1991; Gutzwiller and Marcum 1993, 1997; Dawkins and Woodington 1997; Riffell and Riffell 2002).

Flight-initiation distance (FID) can be used to test the species-confidence hypothesis because this measures perception of risk (Ydenberg and Dill 1986; Gutzwiller and Marcum 1993, 1997; Kramer and Bonenfant 1997; Cooper 1999). Perceived risk, and hence flight distances, should be lower when a bird is approached by a researcher wearing a familiar colour. Previous studies have shown that FID is influenced by the presence or absence of a bright orange hunting vest (Gutzwiller and Marcum 1993, 1997), but other

10.1071/MU03033 0158-4197/04/030267

colours have not been tested. This has implications for biologists that are studying birds, as the colour of their clothing may influence the results they acquire (Riffell and Riffell 2002). Only two studies have explored this hypothesis in the field (Gutzwiller and Marcum 1993, 1997). Both used FID and focused on birds' reactions to an approaching human wearing a bright orange hunting vest. The researchers assumed that birds with orange and red plumage would have decreased FIDs when approached by humans wearing orange vests. These studies were somewhat inconclusive. Three out of the seven species of birds with no red or orange on their bodies seemed to be repelled by the orange hunting vest as they exhibited longer FIDs when it was worn. Birds that did have these colours did not change in their FID when the vest was worn, showing that they were more tolerant of these colours, although they were not attracted to them (Gutzwiller and Marcum 1997).

We measured FID to test the species-confidence hypothesis on Spiny-cheeked Honeyeaters (*Acanthagenys rufogularis*). We tested for the effects of colour *per se* and controlled for the possibility that bright colours may have influenced flight distances simply because bright colours may be more detectable. We chose four colours for this study. Two colours were highly detectable (red and yellow) and two had low detectability (tan and olive green). Each of these categories included a colour that occurred on the birds' body (red and tan) and one that did not (yellow and olive green).

We made the following predictions:

1. We predicted that if the species-confidence hypothesis affects FID, researchers wearing colours that were on the bird would be able to approach significantly closer to birds than researchers wearing colours not on the bird (Gutzwiller and Marcum 1993, 1997). Specifically, birds should fly away at greater distances when approached by researchers wearing yellow and olive green shirts, and at shorter distances when wearing red and tan shirts.
2. We predicted that if detectability alone caused differences in FIDs, shirt colours with the highest detectability (red and yellow) would result in greater FIDs, while shirt colours with low detectability (tan and olive green) would result in decreased FIDs.
3. Birds can discriminate colours in the UV spectrum (McGraw *et al*. 1999; Hart 2001; Hunt *et al*. 2001). We thus predicted that if UV vision in birds was the only factor affecting FID, the T-shirt with the highest UV reflectance would induce greater FIDs than those produced by shirts reflecting less UV.
4. Novelty might also influence responsiveness. We predicted that if the novelty of the colour influenced FIDs, then birds would flee the shirt that was the least common colour in the environment at greater distances than other colours.

Methods

Species selection

This experiment was conducted at the Fowlers Gap Arid Zone Desert Research Station (31°5′S, 142°42′E), New South Wales, Australia. We focused on the Spiny-cheeked Honeyeater because it occurred there at a high population density, and its bright red-tipped bill and tan belly were ideal for our study.

Site selection

We selected two large sites (7.42 ha for Site 1 and 5.15 ha for Site 2), on the basis of habitat type and where the birds were found during a preliminary survey; the sites were away from human activity. During the survey we noted that *A. rufogularis* fed primarily on the yellow flowers of eucalyptus. This species of honeyeater was primarily found singing, foraging or perching in the dry creek bed 'wash' on River Red Gum (*Eucalyptus camalduensis*), and on the Narrow-leaf Hopbush Trees (*Dodonaea viscosa angustissima*) in the sparsely vegetated region of the wash periphery.

Quantifying detectability

As a first approximation of detectability we created 0.6 cm × 0.7 cm colour patches matching the T-shirt colours in Adobe Photoshop™, and inserted them into 14.9 cm × 9.8 cm digital photographs of each habitat type; the dry creek bed ('wash') and the 'open'. These images were presented to 90 UCLA undergraduate students who had no knowledge of the experiment and were simply asked to rank the colours in order of most visible to least visible in each habitat. We measured the UV reflectance of each shirt by hanging the shirts ~30 cm from a long-wave (366 nm) UV lamp. A Pasco CI-9784 UVA light sensor measured reflected UV light from the shirts.

General field methods

Three people conducted the FID study. We always wore khaki field pants and one of the four coloured shirts when conducting trials. To quantify FID we first spotted a bird and then approached at a constant pace (0.7 m s^{-1}). Observers trained to walk with a constant pace across the varied terrain before data collection began. We walked and counted the number of paces until the bird took cover or flew away, and recorded this number. Finally, the number of paces to the original position of the bird were counted and recorded on the data sheet. This was recorded as the starting distance. FID was the difference in paces between starting distance and distance flushed. We also noted the bird's initial behaviour (foraging, perched, singing), and the height of the bird from the ground. Following Blumstein *et al*. (2004), the bird's height in a tree was estimated by holding a pen at arm's length and aligning the tip of the pen with the bird. Observers then rotated the pen 90° so that the tip now was on the ground. The distance on the ground between the pivot point (the point directly below the bird), and the tip of the pen was measured and this was the same as the bird's height in the tree. Again, observers trained with this method until heights were measured accurately.

We aimed to not flush the same birds repeatedly by visiting different parts of our study plots. However, some degree of resampling was possible (see Results). Repeatedly flushing the same subjects could lead to habituation – a decline in responsiveness over time, or sensitisation – an increase in responsiveness over time. Most studies that measure FID use unidentified subjects. In a study of identified subjects (Runyan and Blumstein, in press) individuals varied in the rate of their habituation and some failed to habituate to repeated human exposure. More importantly, Runyan and Blumstein (in press) demonstrated that, even with some moderate degree of resampling, the overall conclusions about factors affecting FID were not changed.

We converted paces to metres and calculated the direct distance between a bird and an approaching human when the bird flushed

Table 1. Number of students ranking shirt colours according to detectability in the two different habitat patches: dry creek bed (*n* = 90) and open area (*n* = 89)

	Dry creek bed		Open area	
	Most visible	Second most visible	Most visible	Second most visible
Yellow	62	24	75	9
Red	24	57	9	41
Tan	0	6	2	36
Olive	4	3	3	3

(Blumstein *et al*. 2004). Because the direct distance to the bird was a diagonal vector with respect to its position in a tree, height in the tree and linear measures of FID and starting distance were used to calculate direct FID. Previous work (Blumstein 2003) demonstrated that the distance at which a human begins to approach a relaxed bird explains significant variation in FID. Thus we also used the 'starting distance' as a covariate in measures of FID. We used these direct measurements in all subsequent analyses.

Population census

Following Gibbons *et al*. (1996), we estimated the number of Spiny-cheeked Honeyeaters on each of our plots by using four point transect censuses. We performed two censuses on both sites, each time by a different researcher to avoid bias due to variation in census conductors. The surveyors then recensused each other's plots. Census points were 50 m apart. Ten point transects were performed in Site 1, and 12 in Site 2. After arriving at a census point we waited 1 min to allow the birds to adjust to our presence, then counted all the birds that could be seen and heard within a 20-m radius over the next 3 min. Any birds seen and heard outside the 20-m radius were recorded as 'outside 20 m'.

Statistical analyses

We used Stat View 5.1 (SAS Institute Inc. 1999) to analyse the data collected. As expected, starting distance significantly explained 46.1% of the variance in FID ($P < 0.0001$). Because of this, starting distance was used as a covariate in subsequent models. We fitted ANCOVA models to determine effects of shirt colour, after explaining variation accounted for by starting distance, on direct FID. We examined the effects of observer and initial behaviour on direct FID by fitting ANCOVA models again with starting distance as a covariate. Another model was fitted to study the effect of the birds' height in a tree on direct FID. In all cases, our α was set to 0.05. Residuals from linear models were examined and were approximately normal.

Results

We estimated that there were 524 Spiny-cheeked Honeyeaters at our two sites (298 in Site 1 and 226 in Site 2). Because we conducted a total of 356 trials we recognise that some of the individuals may have been flushed more than once. To humans, red and yellow are both highly detectable colours. In both habitat types yellow was slightly more detectable than red according to the students surveyed (Table 1). As predicted, tan and olive were much less detectable in both habitat types than the red and yellow.

In contrast to the detectability in the visible spectrum, the UV reflectance of the tan shirts was greatest (35.0% of maximum absorbance). Yellow (22.0%), olive (20.5%), and red (9%) were less reflective in the UV spectrum and therefore they are likely to be less detectable by birds with UV-sensitive vision.

We tabulated the numbers of birds with red and yellow on their bodies using a local field guide (Pizzey and Knight 1997). In Australia, there were significantly fewer birds with red on their bodies than expected, while there were significantly more birds with yellow on their bodies than expected ($P < 0.0001$) (Table 2*a*). When we did the same tabulation for the birds at Fowlers Gap, we found that the numbers of birds with red and/or yellow on their bodies did not differ significantly from the expected values ($P = 0.50$) (Table 2*b*). Thus, red and yellow were equally novel (or equally familiar) at our study site.

We found a significant effect ($P = 0.014$) of shirt colour on direct FID (Fig. 1). *Post hoc* analyses revealed that birds fled at significantly shorter distances when approached by people in red shirts ($P = 0.0008$). There was also a significant interaction between shirt colour and starting distance ($P < 0.0001$). To better understand this interaction, we used four parametric ANCOVA models. We found significant differences in the interaction between starting distance and shirt colour when comparing yellow and olive ($P < 0.0001$), yellow and red ($P < 0.0001$), and yellow and tan ($P = 0.0012$).

Our results are neither obscured nor confounded by two other potentially important variables. There was no effect of height in tree on direct FID, as shown by a regression of these two variables ($R^2 = 0.004$, $P = 0.81$). Similarly, a Kruskal–Wallis test showed that there was no change in initial behaviour of the focal bird on direct FID ($P = 0.79$). In other

Table 2. Frequency of red and yellow in all Australian birds and in birds at Fowlers Gap

'On' and 'Off' indicate whether the colours were found on birds

	Red	Yellow	Total
All Australian birds			
Off	676	616	1292
On	102	162	264
Total	778	778	1556
Birds at Fowler's Gap			
Off	42	38	80
On	11	15	26
Total	53	53	106

words, birds that were foraging fled at the same distance from a given shirt colour as those that were perched or singing.

Discussion

We found that Spiny-cheeked Honeyeaters fled approaching humans in ways that supported the species-confidence hypothesis. Both the main effect and interaction results are consistent with the species-confidence hypothesis. Birds fled at a 23% greater distance when researchers approached in yellow shirts, as opposed to red shirts. That Spiny-cheeked Honeyeaters fled approaching humans at a greater distance implied an increased sensitivity towards yellow, or a higher tolerance towards red. There are various explanations for this phenomenon.

One such explanation would be that red is a relatively rare colour in Australian birds, and therefore it was novel to the honeyeaters, causing them to fly at greater distances. That our focal species had smaller FIDs when approached by humans with red T-shirts suggests that this was not a result of novelty. Furthermore, both red and yellow were colours on birds at Fowlers Gap. For this reason, we can conclude that the Spiny-cheeked Honeyeaters were probably not reacting to red as a novel colour.

Our results are inconsistent with the hypothesis that UV reflectance could affect our results. We predicted that if the UV reflectance of the shirts worn during this experiment influenced FID, then the shirt colour with the greatest UV reflectance would elicit the longest FID. After performing UV-reflectance tests on the shirts worn during the experiment, we found that the tan shirt was the most reflective, followed by yellow, then olive, then red. With these data, we would have predicted that the tan shirt should elicit the greatest FID, but we did not find this.

Another explanation for our results might be that red is a carotenoid pigment (Finger and Burkhardt 1994), and therefore has special signal value (Inouye *et al*. 2001). Carotenoid pigments can be acquired only by direct ingestion and, conceivably, birds might be more sensitive to this colour because it would attract them to food sources or would have special signal value. This, in turn, could influence bird responses to a researcher approaching in a red shirt. If this was the case, it could explain the results that researchers were able to get closest to the Spiny-cheeked Honeyeater when wearing red. However, yellow is also a carotenoid pigment colour (Finger and Burkhardt 1994). Because both red and yellow are carotenoid colours, and our main result was that birds had different FIDs to people wearing red and yellow, the carotenoid hypothesis can be dismissed.

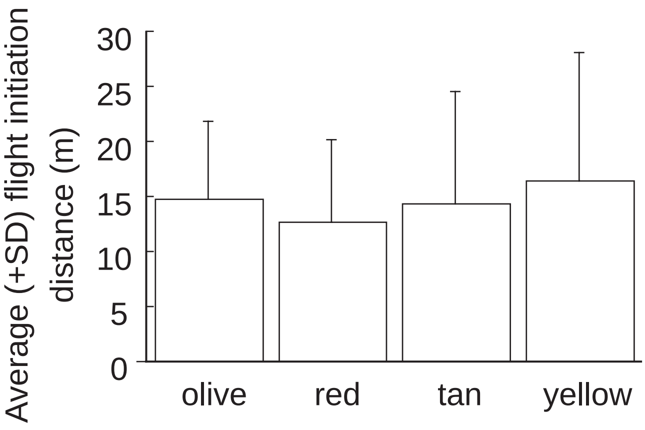

Fig. 1. Bar graph of mean flight-initiation distance for four different colours of T-shirts. There is a significant difference between red and yellow ($P = 0.0140$).

At the outset of this study, we had two main predictions. The first was that birds would react differentially to colours depending on their detectability. To test this, we had two colours that were highly detectable (by humans), and two colours that were not highly detectable. In each of these categories there was a colour that was found on the bird's body and one that was not. If detectability affected FID, then we would have found that the birds would have flushed at greater distances for highly detectable colours such as yellow and red, but have shorter FIDs for the tan and olive green colours. Our data, however, showed that birds flush at significantly shorter distances when the observer was wearing red. Red, although highly detectable, was not found to be as detectable as yellow by humans. If birds, which admittedly have different visual abilities, were to rank detectability similarly, our result may be confounded by differential detectability. Despite the fact that red was very visible in both habitats, FIDs were significantly shorter when wearing red than when wearing the non-detectable colours (tan and olive). Therefore the species-confidence hypothesis may still explain the behaviour of Spiny-cheeked Honeyeaters.

We predicted that birds would be more tolerant of researchers approaching them when the latter wore colours found on the bird's body. Conversely, we predicted that birds would flush sooner when a researcher approached wearing colours not found on the bird's body. Indeed, the Spiny-cheeked Honeyeater seems to be much more tolerant of the colour red, which is found on its beak, than yellow. Although these results may stem from yellow being more visible than red, this study provides evidence that the species-confidence hypothesis can be applied to more than species recognition and mate evaluation (Burley 1986).

The fact that birds exhibit sensitivity and preferences for specific colours has direct ramifications on field studies conducted to survey birds (Cooke 1980; Gutzwiller and Marcum 1993; Gutzwiller *et al*. 1998; Fernández-Juricic *et al*. 2001). These surveys used multiple methods, including FID, to test birds' responses to human impact in parks as well as to predators and perceived risks. When these studies are conducted, the colour of the clothing worn by the surveyor can influence the results. Most studies try to control for this by having researchers wear the same colour clothing (Gutzwiller and Marcum 1993; Gutzwiller *et al*. 1998; Fernández-Juricic *et al*. 2001). Although this controls for differences in colours worn by the researchers, wearing the same colour does not take into consideration how species may react to the colour chosen.

Acknowledgments

We thank the UCLA Department of Organismic Biology, Ecology, and Evolution, the UCLA Office for Instructional Development, and The Lida Scott Brown Ornithology Endowment for generous support of the 2002 Field Biology Quarter expedition to Australia. Research protocols were approved by the University of New South Wales Animal Care and Ethics Committee (permit # 02/08 approved on 5 April 2002). Thanks to Janice Daniel and Aviva Liebert for their help with the statistical analysis, Martin Simon of the UCLA Physics department for his help with the UV-reflectance analysis, and the students of Chemistry 14A and Life Science 1 at UCLA for ranking detectabilities. Finally, we thank an anonymous reviewer whose insightful and extremely constructive comments helped us improve an earlier version of this MS.

References

Blumstein, D. T. (2003). Flight-initiation distance in birds is dependent on intruder starting distance. *Journal of Wildlife Management* **67**, 852–857.

Blumstein, D. T., Fernández-Juricic, E., LeDee, O., Larsen, E., Rodriguez-Prieto, I., and Zugmeyer, C. (2004). Avian risk assessment: effects of perching height and detectability. *Ethology* **110**, 273–285. doi:10.1111/J.1439-0310.2004.00970.X

Burley, N. (1986). Comparison of the band-colour preferences of two species of estrildid finches. *Animal Behaviour* **34**, 1732–1741.

Burley, N., Krantzberg, G., and Radman, P. (1982). Influence of colour-banding on the conspecific preferences of Zebra Finches. *Animal Behaviour* **30**, 444–455.

Cooke, A. S. (1980). Observations on how close certain passerine species will tolerate an approaching human in rural and suburban areas. *Biological Conservation* **18**, 85–88. doi:10.1016/0006-3207(80)90072-5

Cooper, W. E. (1999). Escape behavior by prey blocked from entering the nearest refuge. *Canadian Journal of Zoology* **77**, 671–674. doi:10.1139/CJZ-77-4-671

Dawkins, M. S., and Woodington, A. (1997). Distance and the presentation of visual stimuli to birds. *Animal Behaviour* **54**, 1019–1025. doi:10.1006/ANBE.1997.0519

Fernández-Juricic, E., Jimenez, M. D., and Lucas, E. (2001). Alert distance as an alternative measure of bird tolerance to human disturbance: implication for park design. *Environmental Conservation* **28**, 263–269.

Finger, E., and Burkhardt, D. (1994). Biological aspects of bird colouration and avian colour vision including ultraviolet range. *Vision Research* **34**, 1509–1514. doi:10.1016/0042-6989(94)90152-X

Gibbons, D. W., Hill, D., and Sutherland, W. J. (1996). 'Ecological Census Techniques: a Handbook.' (Cambridge University Press: Cambridge.)

Goforth, W. R., and Baskett, T. S. (1965). Effects of experimental color marking on pairing of captive mourning doves. *Journal of Wildlife Management* **29**, 543–553.

Gutzwiller, K. J., and Marcum, H. A. (1993). Avian responses to observer clothing color: caveats from winter point counts. *Wilson Bulletin* **104**, 628–636.

Gutzwiller, K. J., and Marcum, H. A. (1997). Bird reactions to observer clothing color: implications for distance-sampling techniques. *Journal of Wildlife Management* **61**, 935–947.

Gutzwiller, K. J., Marcum, H. A., Harvey, H. B., Roth, J. D., and Anderson, S. H. (1998). Bird tolerance to human intrusion in Wyoming montane forests. *Condor* **100**, 519–527.

Hart, N. (2001). Variations in cone photoreceptor abundance and the visual ecology of birds. *Journal of Comparative Psychology* **187**, 685–698. doi:10.1007/S00359-001-0240-3

Hunt, S., Cuthill, I. C., Bennett, A. T. D., Church, S. C., and Partridge, J. C. (2001). Is the ultraviolet waveband a special communication channel in avian mate choice? *Journal of Experimental Biology* **204**, 2499–2507.

Inouye, C. Y., Hill, G. E., Stradi, R. D., and Montgomerie, R. (2001). Carotenoid pigments in male House Finch plumage in relation to age, subspecies, and ornamental coloration. *Auk* **118**, 900–915.

Kramer, D. L., and Bonenfant, M. (1997). Direction of predator approach and the decision to flee to a refuge. *Animal Behaviour* **54**, 289–295. doi:10.1006/ANBE.1996.0360

McGraw, K. J., Hill, G. E., and Keyser, A. J. (1999). Ultraviolet reflectance of colored plastic leg bands. *Journal of Field Ornithology* **70**, 236–243.

Metz, K., and Weatherhead, P. (1993). An experimental test of contrasting-color hypothesis of red-band effects in Red-winged Blackbirds. *Condor* **95**, 395–400.

Pepperberg, I. M. (1987). Acquisition of the same/different concept by an African Grey Parrot (*Psittacus erithacus*): learning with respect to categories of color, shape, and material. *Animal Learning and Behavior* **15**, 423–432.

Pizzey, G., and Knight, F. (1997). 'The Field Guide to the Birds of Australia.' (Angus and Robertson: Sydney.)

Riffell, S. K., and Riffell, B. D. (2002). Can observer clothing color affect estimates of richness and abundance? An experiment with point counts. *Journal of Field Ornithology* **73**, 351–359.

Runyan, A., and Blumstein, D. T. (2004). Do individual differences influence flight-initiation distance? *Journal of Wildlife Management*, in press.

SAS Institute, Inc. (1999). 'StatView 5.1.' (SAS Institute, Inc.: Cary, NC.)

Slobodchikoff, C. N., Kiriazis, J., Fischer, C., and Creef, E. (1991). Semantic information distinguishing individual predators in the alarm calls of Gunnison's Prairie Dogs. *Animal Behaviour* **42**, 713–719.

Weatherhead, P., Hoysak, D., Metz, K. J., and Eckert, C. (1991). A restrospective analysis of red-band effects on Red-winged Blackbirds. *Condor* **93**, 1013–1016.

Ydenberg, R. C., and Dill, L. M. (1986). The economics of fleeing from predators. *Advances in the Study of Behaviour* **16**, 229–249.

Manuscript received 21 July 2003, accepted 20 May 2004