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Melanocortin-1-receptor (*MC1R*) variation is not associated with parasite burden in a neotropical bird, the bananaquit (*Coereba flaveola*)

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It has been suggested that selection on melanocortin-1-receptor (*MC1R*) polymorphism, a common cause of melanic colour variation in vertebrates, results from pleiotropic effects of the gene in the immune system. Here we present the first test of whether *MC1R* variation is associated with differences in parasite abundance in a natural population. Bananaquits (*Coereba flaveola*) (Linnaeus, 1758) living on Grenada in the Caribbean exhibit a melanic plumage dimorphism as a result of a mutation in *MC1R*. The proportion of black individuals increases clinally towards the central, wetter parts of the island. We captured bananaquits through the cline and quantified parasite abundances. Avian malaria, feather mites, and mallophaga lice varied significantly in abundance across the cline; however, neither these infections, nor coccidia, nor arboviruses showed overall differences between the morphs. Feather mites tended to be more abundant on black individuals, in areas where the black morph was more common. This may result from differences in microhabitat use by the two morphs. These patterns do not support the idea that *MC1R* variation in itself results in differing susceptibility to parasites. *Biological Journal of the Linnean Society*, 2013, ••, ••–••.

ADDITIONAL KEYWORDS: avian malaria – cline – melanin – pleiotropy.

INTRODUCTION

Throughout the history of evolutionary biology there has been a fascination with polymorphisms (Bates, 1861). Since before the advent of molecular genetics they have represented model systems for studying natural selection in the wild, while simultaneously allowing inference about genotypes (Cain & Sheppard, 1954; Kettlewell, 1973), and providing the first and most resonant examples of evolution occurring in nature (Bates, 1861; Kettlewell, 1973). More recently, significant strides have been made in determining the genetic basis of several prominent polymorphisms (Theron *et al*., 2001; Hoekstra & Nachman, 2003).

Although this new genetic understanding is interesting in itself, it further highlights the fundamental evolutionary question that remains unresolved: what mechanisms are responsible for the maintenance of polymorphisms in natural populations? In some cases, for example where differences in colour provide crypsis, the answer may seem obvious (Kettlewell, 1973; Vignieri, Larson & Hoekstra, 2010), but even then years of study may fail to reveal the whole answer. For example, Brakefield has shown (Brakefield, 1987) that differential predation on adults cannot by itself account for the differences in selection on morphs of the peppered moth (*Biston betularia* Linnaeus, 1758). One possibility is that the obvious phenotype that results from a genetic polymorphism is not itself under selection, but rather there are pleiotropic effects that affect fitness (Chakarov, Boerner & Kruger, 2008; Ducrest, Keller & Roulin, 2008).

Differences in animal colour provide some of the most striking examples of polymorphisms (Theron

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et al., 2001; Hoekstra & Nachman, 2003; Chakarov *et al*., 2008), and investigation of their genetic causes has been facilitated by their simple Mendelian basis (Theron *et al*., 2001; Hoekstra & Nachman, 2003). Mutations in the melanocortin-1-receptor (*MC1R*) gene are a common source of colour polymorphisms (Theron *et al*., 2001; Hoekstra & Nachman, 2003; Rosenblum, Hoekstra & Nachman, 2004). These are sometimes associated with advantages in crypsis (Vignieri *et al*., 2010), but sometimes have little obvious adaptive significance (Wunderle, 1981). This has led to the suggestion that these polymorphisms may be maintained by less obvious pleiotropic effects (Ducrest *et al*., 2008). Importantly, biomedical research suggests that *MC1R* variation may have effects in the vertebrate immune system that are important in parasite resistance (Lipton *et al*., 1999; Catania *et al*., 2004; Loser *et al*., 2010). Previous studies have also documented associations between melanic coloration and both immune system function and parasite burdens in natural populations (Chakarov *et al*., 2008; Gasparini *et al*., 2009; Piault *et al*., 2009; Jacquin *et al*., 2011). However, in these studies the genetic basis of melanic colour variation was unknown. Gangoso *et al*. (2011) have shown an association between *MC1R* genotypes and cellular immunity in polymorphic Eleonora's falcon (*Falco eleonorae* Gene, 1839), but to date there are no published studies of associations between plumage variation arising from *MC1R* polymorphism and parasite burdens. If it is true that variation at the *MC1R* locus affects functional immunity, we would expect different *MC1R* morphs to experience different levels of parasitic infections in natural populations. Here we present the first data on associations between the *MC1R* phenotype and parasites in a natural population.

In the Caribbean, bananaquits (*Coereba flaveola*) (Linnaeus, 1758), occur as two distinct plumage morphs (see Figs S1 and S2) because of a polymorphism in *MC1R* (Theron *et al*., 2001). The wild type ('yellow') is ubiquitous across the region, whereas an all 'black' morph is found only in the southern Caribbean. The black morph is the result of a dominant point mutation in the coding part of the *MC1R* gene (Theron *et al*., 2001). In Grenada morph frequencies exhibit clinal variation: yellow birds are commonest at the northern and southern ends of the island, and the proportion of black birds increases towards the central areas (Wunderle, 1981). In the south the cline is narrow: the proportion of black birds increases from 10 to 90% over a distance of 4 km (Wunderle, 1981; MacColl & Stevenson, 2003). Morph frequencies are strongly associated with variation in climate and habitat. Yellow birds are commonest in the driest, low-lying areas of open woodland, scrub, and suburban habitats, whereas the frequency of black birds increases in wetter areas at higher altitudes where there is dense scrub, woodland, and forest. However, it is important to emphasize that both morphs occur throughout a large part of these climate and habitat transitions, with yellow birds only absent from the wettest areas, where the forest is most developed (MacColl & Stevenson, 2003). Wunderle suggested that differences in plumage colour might be associated with small differences in microhabitat selection within locations (e.g. black birds preferring shady over sunny spots), but given the large variation in climate and habitat through the cline, this is only likely to contribute a small amount to the variance in habitats experienced by the two morphs.

Here, we examine whether the *MC1R* mutation might be associated with differences in parasite abundance between the two plumage morphs. These might come about by two different mechanisms. There could be a direct pleiotropic 'genetic link' (Jacquin *et al*., 2011) between *MC1R* polymorphism and immune system function, resulting in different intrinsic resistance to parasites. Alternatively, the use of different habitats by the morphs might result in different parasite exposure ('exposure hypothesis', Jacquin *et al*., 2011). As the clinal variation in morph frequencies on Grenada is associated with substantial variation in habitats (Wunderle, 1981), which may result in differences in exposure to different parasites, bananaquits on Grenada provide a powerful system for testing both hypotheses in natural conditions. The genetic link hypothesis predicts an overall difference in parasite abundance between the morphs (statistically an effect of 'morph' on parasite abundance). The exposure hypothesis predicts that any difference in parasites between the morphs will vary with location in the cline. An overall variation in parasites across the cline (an association between position in the cline and parasite abundance) would indicate that variation in habitat is associated with variation in exposure for bananaquits in general. Differences between the morphs in their parasites at different locations (statistically an interaction between morph and location) provides a test of the exposure hypothesis.

MATERIAL AND METHODS

Bananaquits were mist-netted at eight sites across the cline (Fig. 1) in south-western Grenada in September 2002 (MacColl & Stevenson, 2003). Birds were weighed (±0.1 g), aged as juveniles, subadults, or adults, using plumage characteristics (Raffaele *et al*., 1998), and blood sampled by brachial venepuncture. The bananaquits were then screened for parasites as follows. (1) The number of feather mites on the primary and secondary feathers of the left

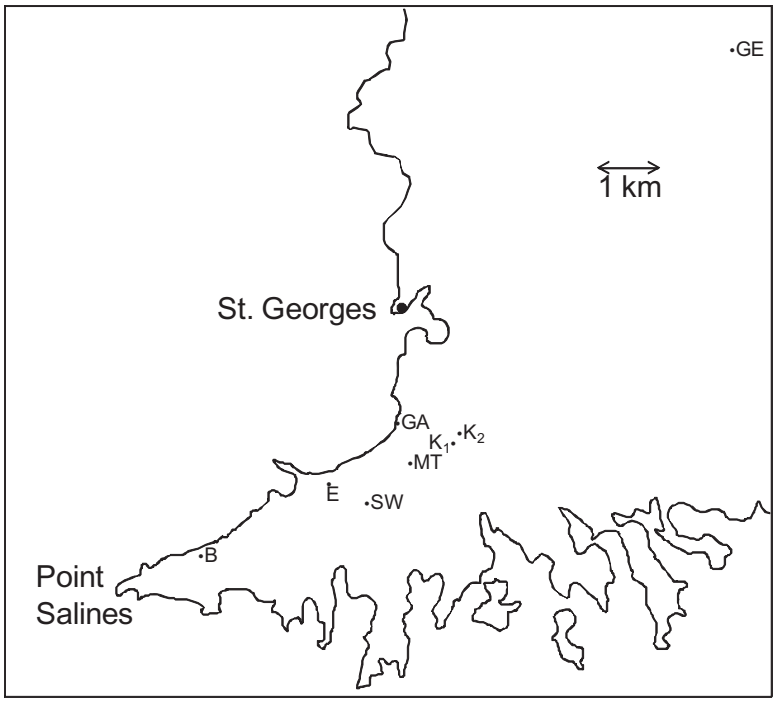

Figure 1. Location of sampling sites in south-western Grenada. In order of distance from Point Salines (the most westerly point on the peninsula): B, Pinguin; E, Blue Horizons; SW, South Winds; MT, Mont Tout; GA, Grand Anse; K_1, golf course south; K_2, golf course north; GE, Grand Etang.

wing was counted with an 8× hand lens (Clayton & Walther, 1997). (2) Each bird was 'dust-ruffled' with pyrethrin powder to collect ectoparasites (Clayton & Walther, 1997), which were then counted under a dissecting microscope. (3) Opportunistically collected faecal samples were weighed and thoroughly mixed in 1.0 ml of saturated saline solution, and an aliquot was loaded into a McMaster counting chamber. The counting grid was searched at a magnification of 100× under a compound microscope to quantify the number of parasite eggs and coccidia per gram of faeces. (4) Genomic DNA was extracted from blood samples and used to sex individuals (Griffiths *et al*., 1998). Infection by *Haemoproteus* or *Plasmodium* was detected by polymerase chain reaction (PCR) amplification of the mitochondrial cytochrome *b* gene extracted from the parasites, and using primers HAEMF and HAEMR2 (Bensch *et al*., 2000). PCR products were separated on 2% agarose gels, and a 525-bp fragment was found in infected individuals. The presence of haematozoan parasites was confirmed by sequencing the positive amplicons. (5) Whole blood was collected into capillary tubes that were sealed, centrifuged at 1000 *g* for 3 minutes, and frozen at –20 °C. These samples were tested for antibodies to alphaviruses (Venezuelan, Eastern, and Western equine encephalitis, and Mayaro viruses) and flaviviruses (St Louis encephalitis and West Nile viruses) using haemagglutination-inhibition tests (Allan & Gough, 1974). Additionally, the legs and feet of bananaquits were examined for lesions or deformations, symptomatic of infection with a virus such as avian pox (Atkinson *et al*., 2005). All fieldwork was carried out under permit from the Grenadan Government.

Variation in parasites was analysed in GENSTAT (Payne *et al*., 2010), using generalized linear models (GLMs). Morph, sex, age, weight, and location in cline (and its interaction with morph) were included as fitted terms. Non-significant terms (judged from the change in deviance upon dropping a term from a model) were removed sequentially from models, which were then refitted to arrive at a minimum adequate model in which all terms were significant (subject to the constraint that main effects marginal to significant interactions had to be retained). It is important to include sex, age, and weight in models because they could potentially confound relationships between morph and parasites. Parasite abundance is often related to sex and age in vertebrates (e.g. Folstad & Karter, 1992; Rousset *et al*., 1996), whereas weight provides a way to control for variation in condition within sex and age classes that could alter susceptibility to infection. The proportion of black birds at a site, estimated from mist-net catches, was used as an estimate of the location of a site in the cline, because this resulted in linear relationships

with measures of parasite abundance. We re-ran the analyses with 'distance from Point Salines' (Fig. 1) as the measure of location, and this made no difference to the results (not shown), but models fitted less well because the relationships between distance and measures of parasite abundance tended to be curvilinear. We interpreted the significance of the fitted term 'morph' as evidence for the 'genetic link hypothesis', and the significance of the morph by location interaction as evidence for the 'exposure hypothesis'.

We have not genotyped the birds in this study at the *MC1R* locus, and therefore as the 'black' allele is dominant for the effect it has on colour, we do not know whether black birds were homozygous or heterozygous at the *MC1R* locus. In our analyses we have made the assumption that any effect of the 'black' allele on parasite resistance is also dominant. If the effects of the two alleles on the immune system are in fact additive this would not matter, as we would still expect an overall difference in the parasite burdens of black versus yellow birds. The assumption we have made would only matter if there is a reversal of dominance of the black and yellow alleles in the effect they have on the immune system. This seems unlikely, given that the black allele results from a coding mutation that means that the melanocortin-1 receptor becomes constitutively active (Theron *et al*., 2001).

RESULTS

We captured 204 bananaquits, of which 92 were of the black morph. The proportion of the black morph varied between 3 and 100% across the eight netting sites (MacColl & Stevenson, 2003). Not all birds were assayed for all parasites because of time and other constraints (Table 1). Feather mites on black birds increased with the percentage of black birds at a site, and tended to be more abundant than on yellow birds, for which there was no relationship between mite abundance and the percentage of black birds at a site (Fig. 2A; Table 1). The only other ectoparasites found were mallophaga lice, and their abundance increased with the percentage of black birds at a site (Fig. 2B; Table 1).

Faecal samples were obtained from 39 birds that were well distributed across the cline, and between black and yellow morphs (21 and 18 samples, respectively). Only coccidial oocysts (*Isospora* sp.) were

Table 1. Results of generalized linear models to test the relationship between the burdens of different parasites in all bananaquits, and relevant fitted terms

Fitted terms error structure, link function, and statistic	Ln (feather mites + 1) normal, identity, F	Mallophaga lice Poisson, logarithm, F	Ln (coccidia + 1) normal, identity, F	Haematozoa binomial, logit, χ^2	Putative pox infection binomial, logit, χ^2
Sample size	184	178	38	180	200
Sex					
statistic	1.01	17.29	0.41	0.16	0.29
d.f.	1, 179	1, 175	1, 36	1	1
significance	0.32	< 0.001	0.53	0.69	0.59
Age class	0.18	2.46	2.67	2.19	0.42
	1, 178	2, 173	2, 35	2	2
	0.84	0.09	0.08	0.11	0.66
Weight	1.21	0.10	0.01	3.52	0.88
	1, 179	1, 174	1, 36	1	1
	0.27	0.76	0.91	0.06	0.35
Position in cline	14.79	83.97	0.38	40.59	0.00
	1, 181	1, 175	1, 36	1	1
	< 0.001	< 0.001	0.55	< 0.001	0.99
Morph	3.40	0.03	0.01	0.68	1.73
	1, 181	1, 174	1, 36	1	1
	0.07	0.86	0.91	0.41	0.19
Location × morph	23.20	0.19	1.70	0.17	0.37
	1, 180	1, 173	1, 34	1	1
	< 0.001	0.66	0.20	0.68	0.54

The table gives statistics for significant terms in the minimum adequate model (MAM) after the elimination of non-significant terms, and for non-significant terms on being dropped from the MAM.

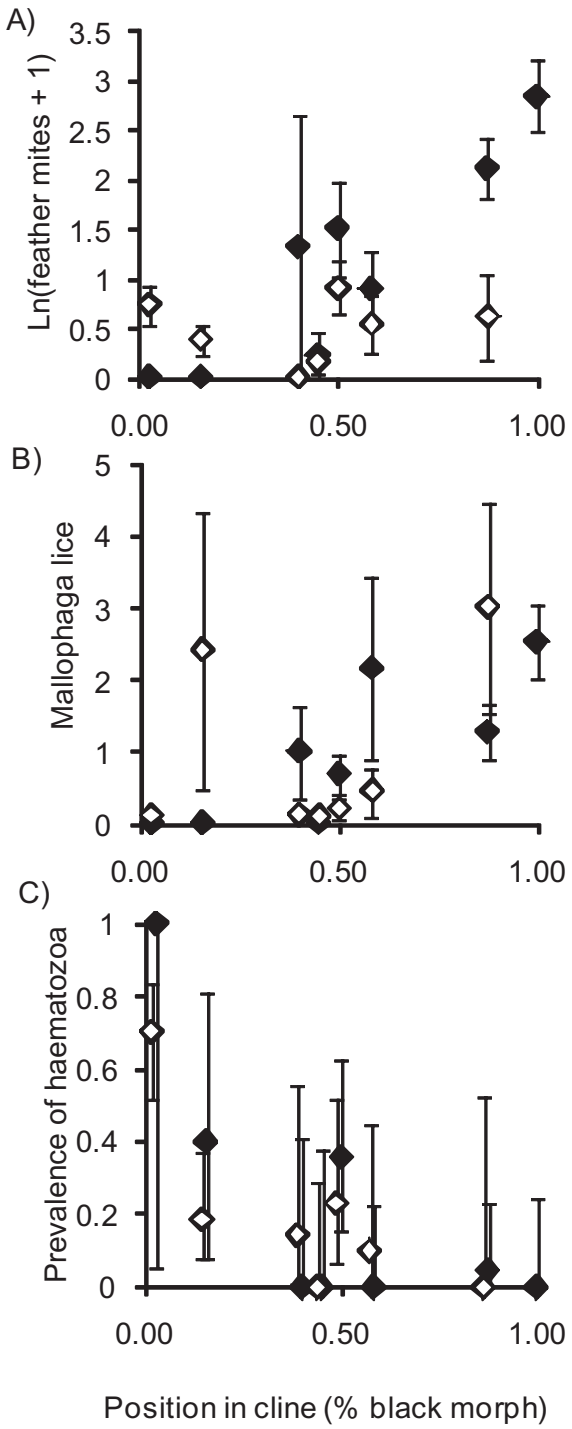

Figure 2. Variation in parasites of black (filled symbols) and yellow (open symbols) bananaquit morphs in relation to location in the plumage cline (proportion of black individuals at the site) in south-western Grenada: A, mean (±SE) of ln(number of feather mites); B, mean (±SE) mallophaga lice on birds; C, prevalence (±95% binomial confidence interval) of haematozoan blood parasites (*Haemoproteus* and *Plasmodium* spp.).

found in the faeces. Their number did not vary with location in the cline, with plumage morph, or with their interaction (Table 1).

Overall, 22% of 180 sampled birds were positive for haematozoa. Sequencing of a subsample of birds revealed that both *Haemoproteus* and *Plasmodium*-spp. were present. The prevalence of haematozoan blood parasites did not differ between the morphs, but was lower at sites where black birds were relatively more common (Fig. 2C; Table 1).

Fifty bananaquits from across the cline tested negative for all six viruses. A single (black) bananaquit was positive for both Eastern equine encephalitis (EEE) and Venezuelan equine encephalitis (VEE), probably as a result of cross reactivity. It is more likely that the bird was infected with EEE. Of 200 birds examined, 7% had avian pox-like deformations on the feet and legs. There was no difference between the morphs, and no relationship with position in the cline (Table 1).

DISCUSSION

There was no overall difference in parasite burdens between the morphs for any of the parasites that we recorded, from ectoparasites to viruses. Therefore, we cannot reject the null hypothesis of no genetic link between *MC1R* variation and parasite resistance in bananaquits. Other recent studies have found associations between the extent of melanic coloration in birds and measures of immune function, and/or parasite infections (Chakarov *et al*., 2008; Gasparini *et al*., 2009; Jacquin *et al*., 2011), but have not related this to variation in *MC1R*, with one exception. Gangoso *et al*. (2011) found an association between *MC1R* genotypes and cellular immunity in *Falco eleonorae*, but the functional relevance of their (phytohaemagglutinin) assay is unknown, and they did not look at variation in parasites among morphs. Our results suggest that *MC1R* variation in itself does not result in differences in susceptibility to a wide range of parasites under natural conditions.

We did document a habitat-specific difference between the morphs in the numbers of feather mites. This supports the exposure hypothesis (Jacquin *et al*., 2011), i.e. that melanic individuals may contract different infestations than non-melanic ones, as a consequence of the habitats that they favour. One possible reason for the differences in numbers of feather mites is that the morphs represent different microclimates for the mites. Black bananaquits spend more time in the shade (Wunderle, 1981), and it is possible that this makes them preferable as hosts for the mites, which are known to be sensitive to temperature and humidity (Proctor, 2003).

There was substantial variation in parasites through the plumage cline. Ectoparasite variation may have resulted from variation in environmental suitability (e.g. temperature and humidity; Proctor, 2003). Variation in the prevalence of haematozoan infections may result from the ecology of their vectors. The high prevalence at the two sites closest to the sea reflects our experience of anthropophilic mosquitoes at these sites. One Caribbean mosquito species (*Deinocerites cancer* Theobald, 1901) breeds in land crab burrows, which are more common close to the sea. It is not known whether this mosquito is a vector of avian haematozoans, but it is known to harbour West Nile virus in Florida (Hribar *et al*., 2003), which suggests that it is ornithophilic.

In summary, parasite burdens do not co-vary with *MC1R* phenotype in bananaquits, and it therefore seems unlikely that selection mediated by the parasites we detected can help to maintain the *MC1R* polymorphism in this species. It remains possible that there are differences between the morphs in their susceptibility to bacterial parasites, especially to feather-degrading bacteria. It has been demonstrated that melanin in feathers prevent feather wear by abrasion, and may inhibit the effects of feather-degrading bacteria (Gunderson, 2008). However, we did not notice any obvious differences in the plumage condition of black and yellow birds in any part of the cline, and in any case there is very little difference between the morphs in the extent of melanization in wing and tail feathers, so it is especially unlikely that the morphs experience differences in feather wear for these most functionally important feathers.

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REFERENCES

Allan WH, Gough RE. 1974. Standard Hemagglutination test for Newcastle disease. 1. Comparison of macro and micro methods. *Veterinary Record* **95:** 120–123.

Atkinson CT, Lease JK, Dusek RJ, Samuel MD. 2005. Prevalence of pox-like lesions and malaria in forest bird communities on leeward Mauna Loa Volcano, Hawaii. *Condor* **107:** 537–546.

Bates HW. 1861. Contributions to an insect fauna of the Amazon valley. Lepidoptera: heliconidæ. *Transactions of the Linnean Society of London* **23:** 495–566.

Bensch S, Stjernman M, Hasselquist D, Ostman O, Hansson B, Westerdahl H, Pinheiro RT. 2000. Host specificity in avian blood parasites: a study of *Plasmodium* and *Haemoproteus* mitochondrial DNA amplified from birds. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267:** 1583–1589.

Brakefield PM. 1987. Industrial melanism: do we have the answers? *Trends in Ecology & Evolution* **2:** 117–122.

Cain AJ, Sheppard PM. 1954. Natural selection in Cepaea. *Genetics* **39:** 89–116.

Catania A, Gatti S, Colombo G, Lipton JM. 2004. Targeting melanocortin receptors as a novel strategy to control inflammation. *Pharmacological Reviews* **56:** 1–29.

Chakarov N, Boerner M, Kruger O. 2008. Fitness in common buzzards at the cross-point of opposite melanin-parasite interactions. *Functional Ecology* **22:** 1062–1069.

Clayton DH, Walther BA. 1997. Appendix C. Collection and quantification of arthropod parasites of birds. In: Clayton DH, Moore J, eds. *Host-parasite evolution. General principles and avian models*. Oxford: Oxford University Press, 419–440.

Ducrest AL, Keller L, Roulin A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology & Evolution* **23:** 502–510.

Folstad I, Karter AJ. 1992. Parasites, bright males, and the immunocompetence handicap. *American Naturalist* **139:** 603–622.

Gangoso L, Grande JM, Ducrest AL, Figuerola J, Bortolotti GR, Andres JA, Roulin A. 2011. MC1R-dependent, melanin-based colour polymorphism is associated with cell-mediated response in the Eleonora's falcon. *Journal of Evolutionary Biology* **24:** 2055–2063.

Gasparini J, Bize P, Piault R, Wakamatsu K, Blount JD, Ducrest AL, Roulin A. 2009. Strength and cost of an induced immune response are associated with a heritable melanin-based colour trait in female tawny owls. *Journal of Animal Ecology* **78:** 608–616.

Griffiths R, Double MC, Orr K, Dawson RJG. 1998. A DNA test to sex most birds. *Molecular Ecology* **7:** 1071–1075.

Gunderson AR. 2008. Feather-degrading bacteria: a new frontier in avian and host-parasite research? *Auk* **125:** 972–979.

Hoekstra HE, Nachman MW. 2003. Different genes underlie adaptive melanism in different populations of rock pocket mice. *Molecular Ecology* **12:** 1185–1194.

Hribar LJ, Vlach JJ, Demay DJ, Stark LM, Stoner RL, Godsey MS, Burkhalter KL, Spoto MC, James SS, Smith JM, Fussell EM. 2003. Mosquitoes infected with West Nile virus in the Florida Keys, Monroe County, Florida, USA. *Journal of Medical Entomology* **40:** 361–363.

Jacquin L, Lenouvel P, Haussy C, Ducatez S, Gasparini J. 2011. Melanin-based coloration is related to parasite intensity and cellular immune response in an urban free living bird: the feral pigeon Columba livia. *Journal of Avian Biology* **42:** 11–15.

Kettlewell B. 1973. *The evolution of melanism: the study of a recurring necessity*. Oxford: Clarendon Press.

Lipton JM, Zhao H, Ichiyama T, Barsh GS, Catania A. 1999. Mechanisms of anti-inflammatory action of alpha-MSH peptides – in vivo and in vitro evidence. *Annals of the New York Academy of Sciences* **885:** 173–182.

Loser K, Brzoska T, Oji V, Auriemma M, Voskort M, Kupas V, Klenner L, Mensing C, Hauschild A, Beissert S, Luger TA. 2010. The neuropeptide alpha-melanocyte-stimulating hormone is critically involved in the development of cytotoxic CD8(+) T cells in mice and humans. *PLoS ONE* **5:** e8958.

MacColl ADC, Stevenson IR. 2003. Stasis in the morph ratio cline in the Bananaquit on Grenada, West Indies. *Condor* **105:** 821–825.

Payne R, Murray D, Harding S, Baird D, Soutar D. 2010. *Introduction to genstat for Windows*. Hemel Hemstead: VSN International.

Piault R, Gasparini J, Bize P, Jenni-Eiermann S, Roulin A. 2009. Pheomelanin based coloration and the ability to cope with variation in food supply and parasitism. *American Naturalist* **174:** 548–556.

Proctor HC. 2003. Feather mites (Acari: Astigmata): ecology, behavior, and evolution. *Annual Review of Entomology* **48:** 185–209.

Raffaele H, Wiley J, Garrido O, Keith A, Raffaele J. 1998. *Birds of the West Indies*. London: Christopher Helm.

Rosenblum EB, Hoekstra HE, Nachman MW. 2004. Adaptive reptile color variation and the evolution of the Mc1r gene. *Evolution* **58:** 1794–1808.

Rousset F, Thomas F, Demeeus T, Renaud F. 1996. Inference of parasite-induced host mortality from distributions of parasite loads. *Ecology* **77:** 2203–2211.

Theron E, Hawkins K, Bermingham E, Ricklefs RE, Mundy NI. 2001. The molecular basis of an avian plumage polymorphism in the wild: a melanocortin-1-receptor point mutation is perfectly associated with the melanic plumage morph of the bananaquit, *Coereba flaveola*. *Current Biology* **11:** 550–557.

Vignieri SN, Larson JG, Hoekstra HE. 2010. The selective advantage of crypsis in mice. *Evolution* **64:** 2153–2158.

Wunderle JM. 1981. An analysis of a morph ratio cline in the bananaquit (*Coereba flaveola*) on Grenada, West Indies. *Evolution* **35:** 333–344.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. An adult black morph of *Coereba flaveola* from Grenada. ©Andrew MacColl.
Figure S2. A wild-type or yellow morph of *Coereba flaveola* from Grenada. ©Andrew MacColl.