Variation in haematozoan parasitism at local and landscape levels in the red-billed quelea *Quelea quelea*

Kate L. Durrant, Jennifer L. Reed, Peter J. Jones, Martin Dallimer, Robert A. Cheke, Andrew N. McWilliam and Robert C. Fleischer

K. L. Durrant (correspondence), J. L. Reed and R. C. Fleischer, Genetics Program, Smithsonian Institution, 3001 Connecticut Ave., NW, Washington, DC 20008 USA. E-mail: k.l.durrant@sheffield.ac.uk. – P. J. Jones Institute of Evolutionary Biology, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK. – M. Dallimer, BIOME Group, Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, UK. – R. A. Cheke, Natural Resources Institute, University of Greenwich at Medway, Chatham Maritime, Chatham, Kent ME4 4TB, UK. – A. N. McWilliam, The Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH UK. – Present address of KLD: Department of Animal and Plant Sciences, The University of Sheffield, Sheffield S10 2TN, UK.

The red-billed quelea *Quelea quelea*, one of the most abundant birds in the world, presents two fundamental conundrums that we investigate here with a novel approach using blood parasite assemblages at two spatial scales, landscape and individual. The quelea of southern Africa *Q. q. lathamii* are split by a hypothesized migratory divide, where birds follow rain fronts in one of two directions (NW or SE). This divide is not detectable in the host population using microsatellite data, and here we show that it is also not apparent from our large-scale phylogeographical analyses of the haematozoan parasite. At a finer scale, the colourful and variable breeding plumage of male red-billed quelea has not previously shown a correlation with predictors of quality, as it does in many other bird species. The male's breeding plumage is partially based on carotenoid colouration, the quality of which has been correlated with haematozoan infection in other bird species. However, we found no correlation between intensity of male carotenoid colouration and haematozoan infection. Our results do not contradict the hypothesis that male breeding plumage in this species serves to identify individuals rather than to indicate quality. Finally, we recovered the greatest number of haematozoan lineages from any phylogenetic survey of a single host species to date. Understanding the reasons for the extreme diversity of parasite lineages in this species may assist in explaining the success of the red-billed quelea in anthropogenic landscapes.

The red-billed quelea *Quelea quelea* is one of the most abundant bird species in the world, and is a major agricultural pest in some regions of Africa. It also serves as a food source for humans living in the sub-Saharan region (reviewed in Elliot 1989). We investigated two unusual aspects of the biology of this species using their haematozoan parasite assemblages as markers at two spatial scales. At the landscape scale, the distribution of haematozoa across quelea populations may provide evidence of the migratory divide postulated for this species. At the local scale, an investigation of haematozoan prevalence in individual males may shed light on

the apparent lack of correlation between bright breeding plumage and indicators of male quality.

In turn, the movements of hosts at both the large and the small scales affect the distribution of their parasites. Important factors at the landscape level include dispersal and population structure of hosts and vectors, while individual host characteristics such as immunocompetence, condition, and natural and sexual selection affect parasite dispersal and population structure on the local scale.

Red-billed quelea males produce elaborate and variable breeding plumage. The crown and throat can

vary from pale to deep yellow or pale pink to deep maroon, the belly from grey to buff suffused with yellow or red, and the facial mask from black to white (Ward 1966, Dale 2000). It has been suggested that despite the breeding plumage colour being partly carotenoid-based, it primarily aids mutual identification in densely populated breeding colonies (Dale 2000, Dale et al. 2001). Physical condition was not found to correlate with the intensity of colour produced by males, but parasitism or disease status was not examined in Dale's (2000) study. Parasite-mediated sexual selection, first proposed by Hamilton and Zuk (1982), implies that individuals that are better able to resist parasites, and are in better health, may advertise this with more elaborate ornaments. An individual's health and condition may be reflected in the intensity of carotenoid-based colours that the individual can produce (Houde and Torio 1992, Brawner et al. 2000, McGraw and Ardia 2003, Saks et al. 2003). Carotenoid colours are thought to be costly to produce and are therefore 'honest' signals of quality (reviewed in Olson and Owens 1998, Møller et al. 2000). For example, male greenfinches *Carduelis chloris* that produced more intense yellows also showed a stronger immune response to a novel antigen and were in better overall health (Saks et al. 2003). Furthermore, a correlation has been established between haematozoa infection, lessened carotenoid colour intensity and reduced local survival rates in the great tit *Parus major* (Hõrak et al. 2001).

We expected there would be a correlation in male red-billed quelea between a quality indicator and elaborate breeding plumage, as has been found in so many bird species to date (reviewed in Olson and Owens 1998, Møller et al. 2000). We predicted that males with less intense carotenoid colouration would have a higher prevalence of haematozoa infection, a correlation that has not previously been tested for in this species. Determining this would inform whether the brightness of plumage is a signal of quality. This in turn may affect parasite distribution in that lower-quality males not selected as mates may have different movement patterns from breeding males.

Given their intimate association, parasites and their hosts may display concordant phylogeographic patterns (reviewed in Criscione et al. 2005). If the parasite is a fast-evolving pathogen, it may also provide information on the structure of its host population in recent time that would be impossible to obtain by other methods (Biek et al. 2006).

The red-billed quelea engages in a rapid and itinerant breeding strategy, moving in response to rainfall patterns (Ward 1971, Jones 1989a). Migrations of the southern African race of red-billed quelea *Q. q. lathamii* are influenced by two rain fronts that approach from either end of a roughly NW-SE axis across the subcontinent. As a result, the pattern of south-easterly and north-westerly movements adopted by quelea are expected to be mirror-images, with the potential to create a geographical split in the population (Dallimer and Jones 2002). This directional migration orientation was tested using Emlen orientation funnels, and individual birds were found to have distinct preferences for orienting either north-west or south-east, consistent with the hypothesis of a migratory divide in this species (Dallimer and Jones 2002). Limited evidence from banding data also indicated cohesiveness of quelea flocks (Jones 1989b). However, genetic evidence for this migratory divide was not apparent in an analysis of population structure using microsatellites (Dallimer et al. 2003). Because divergence in parasite sequences can track the divergence of the host sequences, albeit at a slightly slower rate in some loci (Ricklefs and Fallon 2002), parasites may show structure in their population concordant with the migratory divide observed indirectly in the field in their hosts. Therefore, our second aim was to determine if there was any phylogeographic structure in the haematozoan parasite assemblage of the red-billed quelea, and if so, whether this correlates with the postulated migratory divide.

Methods

Sample collection and processing

Blood or tissue samples (n = 277) were obtained from red-billed quelea collected in southern Africa by R. A. Cheke, M. Dallimer, P. J. Jones and A. McWilliam over several years (1997–2005). Birds were killed as part of governmental pest-control programmes and collected with the relevant agencies' permission. Samples originated from locations in three main regions that we established post-hoc, central (CEN – seven sites, n = 169) encompassing the centre of the postulated migratory divide, north-west (NW – six sites, n = 35) and south-east (SE – seven sites, n = 73) on either side of this divide (Fig. 1). Most birds were sampled from nesting colonies during the breeding season (coinciding with the wet season in southern Africa, approximately October to March), although samples at two central sites (Lake Manyane, LM, n = 9 and Kotolaname, KN, n = 51) were taken from non-breeding birds at roosts during the dry season.

We extracted host and parasite DNA from the samples using the manufacturer's protocols supplied with Qiagen DNeasy kits. We verified successful DNA extractions for every sample by amplifying a small fragment of avian cytochrome *b* DNA using primers cytb-2RC and cytb-wow (268bp; Dumbacher et al. 2003).
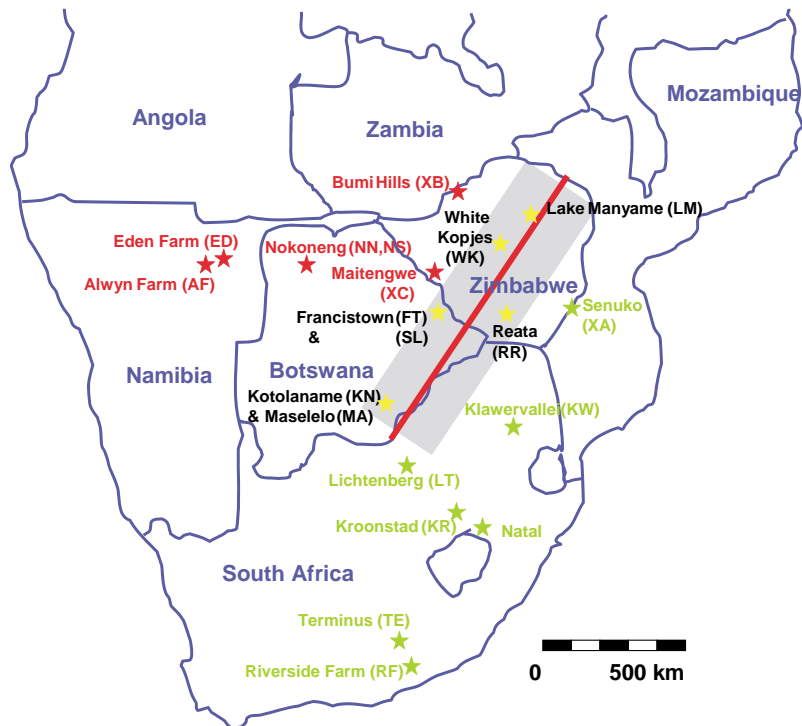
Fig. 1. The sampling locations of red-billed quelea used in this study. We determined three regions pos-hoc: north-west (NW) with red stars, central (CEN) with yellow stars, and south-east (SE) with green stars. The colours coordinate with the haplotype locations on Fig. 3. The grey shaded area and red line denotes the centre of the hypothesized migratory divide for the quelea populations.

We screened each potential host's sample three times using different primer sets designed to amplify the blood parasites *Plasmodium* and *Haemoproteus*. Although our primer sets are sensitive to small quantities of parasite DNA (Beadell and Fleischer 2005), amplification may still be difficult, whether due to the parasite DNA being swamped with host DNA, degraded, or present in low copy. Therefore, the use of more than one set of screening primers ensures that a true picture of infection prevalence is obtained (Beadell et al. 2004, Beadell and Fleischer 2005). Short fragments were initially amplified using three primer sets: F2/R2 (132 bp, mtDNA cyt. *b*); 850F/1024R (167bp, mtDNA COIII) (see: Beadell et al. 2004); and 213F/372R (160bp mtDNA cyt. *b*) (Beadell and Fleischer 2005). PCR reactions typically followed conditions developed for 'ancient' DNA, to increase the probability of successful amplification of possibly degraded samples or samples with low levels of parasitemia (Fleischer et al. 2000). Multiple positive and negative controls were included in every PCR reaction. Samples that produced UV-visible bands after electrophoresis of the PCR product, indicating infection with one or more haematozoa, were then re-amplified with primers designed to target longer

fragments of mtDNA cyt *b*: 3760F/4292R (574bp); or a combination of either F1 or F3 with 4292R (475bp or 336bp, respectively) (Beadell et al. 2004); or FIFI/FIRI (423 bp) or a combination of FIFI with another reverse primer (Ishtiaq et al. 2006). The use of multiple primers that amplify a variety of haematozoan mitochondrial haplotypes likely reduced bias towards a particular region within southern Africa.

PCR products that produced the largest fragment for an infected sample were purified using Qiaquick kits (Qiagen) and bidirectionally sequenced on an ABI 3100 Sequencer (Applied Biosystems). Sequences were as-sembled, aligned, and edited using the program Sequencher v. 4.1. The sequences returned were of high quality and there were no gaps in the resulting alignments.

In order to identify the genus of haematozoan present (*Plasmodium* or *Haemoproteus)*, we used both the phylogenetic alignment of sequences and the results of a restriction enzyme test on positive amplifications utilizing the 213F/372R primer pair (Beadell and Fleischer 2005). This resulted in identifying the genus of haematozoan in most infected samples, including mixed infections. Due to the problems of low PCR amplification inherent in these types of haematozoan

phylogenetic studies (Järvi et al. 2002, Beadell et al. 2004, Ricklefs et al. 2005), not all infected samples were identified to genus.

Phylogenetic analyses

Samples with the longest sequences available, greater than 186 bp, were used to estimate parasite phylogenetic relationships. The average sequence length was 501 bps. The program MacClade v. 4.06 (Maddison and Maddison 2000) was used to detect unique haplotypes; one or more base differences between sequences defined a unique haplotype, and we combined unique haplotypes from all three regions into a single dataset. We reconstructed a phylogenetic tree using a neighbour-joining method, distance criterion, and a Kimura-2 parameter evolutionary model in Paup* (Swofford 1999). Alternative tree-building methods did not produce substantially different tree topologies. Known examples of *Haemoproteus* (GenBank accession number: AY714137) and *Plasmodium* (GenBank accession number: AY733088) species were included in the tree to identify genera, when we recovered a sequence that matched a recorded GenBank sequence, the name of the recorded sequence was included in the tree (*Plasmodium relictum* GRW4, accession number AY733090). We rooted the tree with mammalian *Plasmodium* cyt *b* sequences (GenBank accession numbers: AY069614, AF069624, AF055587, AY099051, AY283019 and AF069610), following the phylogeny developed by Perkins and Schall (2002). Bootstrap values were calculated for the final tree based on a neighbour-joining method with 1000 replicates.

Statistical analyses

Basic statistics were performed using SPSS v. 10.0.5. Chi-square tests were used to test differences in prevalence between regions and differences in the type of infections detected in each region (*Plasmodium* spp., *Haemoproteus* spp., or a mixture of more than one lineage). The program EstimateS v 7.5 (Colwell 2005) was used to calculate the Chao2 estimate of species richness: it is particularly appropriate for both presence/absence data and small sample sizes. We used this statistic to determine the total number of haematozoan lineages that could be expected from our available sequence data. We also used this program to calculate Shannon's diversity indices to describe the relative diversity of samples between regions.

Plumage colour analyses

For the subset of samples obtained during 2004 and 2005, we had photographic data along with a sample of tissue and blood. All of these samples were taken during the breeding season, and we analysed plumage colour data for all males for which we had both a photograph and a blood sample. Colour photographs of sampled males were analyzed using Adobe Photoshop v. 7.0. Each photograph was set up with samples lined up in identical poses (up to 7 per image) on a standard Kodak R 27 grey background, and Kodak Q13 colour standards and grey scales were included at the top and bottom of each image. A Minolta X700 camera with a 50 mm lens was held at a standard distance of 0.7 m vertically above the specimens. Photographs were either taken in the shade or indoors. Once images were opened in the Photoshop program, the grey background on each photograph was adjusted so that the brightness was 50, and the image resolution was reduced so that it was equivalent to 5 pixels per cm. Using the 'colour picker' tool, an average sample of plumage 1 cm^2 was analysed. Using the HSB colour scale, hue was measured on the red and yellow colour standards (pure yellow $=60$, pure red $=0$). Luminosity scores were taken for the pure white colour block, and K scores (percentage of black) were taken for the pure black squares.

Plumage colour scores were measured using the colour picker tool and averaging the scores from four colour picks from three areas of plumage. The three areas measured were the belly (the widest part of the bird as it lay on its back, measuring non-ruffled outer feathers), the throat, and the mask on the face. Belly and throat plumage used the hue score to give an indication of where the colour lay between yellow and red. The mask score was derived from K, the percentage of black in a colour pick, and was corrected for the brightness of the image, as measured by the K score of the black colour block on the image itself. Thus each male bird produced three scores, belly hue, throat hue and mask blackness, each of which could be compared to their parasite infection status.

Endler (1990) proposed five sources of error in visual assessment of animal colouration that we have attempted to avoid. (1) Subjectivity: removed by the use of computer-based scoring methods and a single scorer that produced multiple numerical hue scores that could be averaged. (2) Error from variation in colour surrounding the area of interest: removed by the use of the focused colour-picker tool and individual pixel targeting. (3) Error introduced by variation in lighting: minimised by photography under similar lighting conditions and eliminated by using a standard background for all photos and adjusting the brightness of

the resulting image in Adobe Photoshop v. 7.0. (4) Individual variation in human visual perception: removed by using a single camera, film type, computer and monitor and by relying on an average of multiple computer-generated colour scores obtained by a single scorer. (5) Differences in the visual perception of the study organism and humans: while this will always remain somewhat difficult to address, a meaningful approximation has been achieved with human perception of carotenoid-based plumage colours, the primary concern of our analyses. See Hill (1998) for a review of the correlations of house finch *Carpodacus mexicanus* carotenoid-based plumage colour scores and their ability to predict patterns of mate-choice, mortality, diet responses, and most importantly for our study, parasitism. Furthermore, UV-visible colouration is not a major concern for our analyses as most carotenoid pigments (especially red and yellow) reflect light only in the visible spectrum (Moss and Weeden 1976).

Results

Prevalence of haematozoa

Of 277 red-billed quelea screened for haematozoan parasites, 175 (63.18%) were infected with *Plasmodium*, *Haemoproteus*, or both parasite genera. Parasite lineages, their overall prevalence and their prevalence by region may be found in Table 1. Prevalence was significantly lower in the central (CEN) region compared to the NW or SE regions ($\chi^2 = 43.14$, df = 2, P = <0.001; Fig. 2a). This difference was also apparent when the non-breeding birds were removed from the dataset ($\chi^2 = 23.97$, df = 2, P = <0.001). A χ^2-test of differences in the relative prevalence of infection types across the three regions was non-significant (*Plasmodium* vs. *Haemoproteus* vs. mixed infections: Fig. 2b).

Phylogeography of haematozoa

Of 175 quelea that were infected with a haematozoan, 92 yielded sequence data of sufficient length to be included in the phylogenetic analysis. There were 16 unique *Haemoproteus* haplotypes and 17 unique *Plasmodium* haplotypes recovered. New sequences have been deposited in the GenBank International Nucleotide Sequence Database (Accession numbers: EF117202 – EF117234). Additionally, a single sequence was recovered matching a known *Plasmodium relictum* lineage, GRW4. The Chao2 (S_2^*) estimate of species richness was greater than the number of lineages actually detected for both *Plasmodium* ($S_{obs} = 18$, $S_2^* = 30.1$, SD = 9.73, 95% CI = 21.03–66.28) and *Haemoproteus* ($S_{obs} = 16$, $S_2^* = 52$, SD = 33.41, 95% CI = 23.68–184.73). It should be noted for the *Haemoproteus* lineage estimate especially, that the standard deviation and confidence intervals are large, and that there is much variability.

These sequences were assembled into a phylogenetic tree. The resulting tree underwent a bootstrap analysis using the neighbour-joining method, replicated 1000 times (Fig. 3). Examination of this tree revealed no distinct geographic structuring of the haematozoan haplotypes detected in the red-billed quelea. Three haplotypes (*Haemoproteus* H6 and H11, and *Plasmodium* P12) were found in all three regions, and three more haplotypes (*Haemoproteus* H2 and H4, and *Plasmodium* P16) were found in the two most geographically separated regions, north-west and southeast. There is a potential clade in the tree that includes haplotypes only found in the south-east and central regions, but it also includes a sequence that matches with a known *Plasmodium relictum* lineage (GRW4) that has a worldwide distribution (Beadell et al. 2006). There is also a well-supported clade that consists of four *Haemoproteus* lineages (H13–H16) from only the north-west and central regions. Generally, however, geographic structuring is weak.

The diversity of haematozoan lineages differed between the three regions, NW Shannon's diversity index = 2.37 (SD = 0.06, 95% CI = 2.34–2.40), CEN Shannon's diversity index = 2.96 (SD = 0.02, 95% CI = 2.95–2.97), SE Shannon's diversity index = 2.11 (SD 0.03, 95% CI = 2.10–2.12). Although the prevalence for the central region was significantly lower

Table 1. Prevalence of haematozoan infections in three regions of southern Africa, NW = north west, CEN = central (encompassing the centre of the migratory divide in the host species quelea), and SE = south east. Undetermined lineage infections are positive results obtained from multiple screens that were unable to have their genus determined due to poor PCR amplification (see Methods).

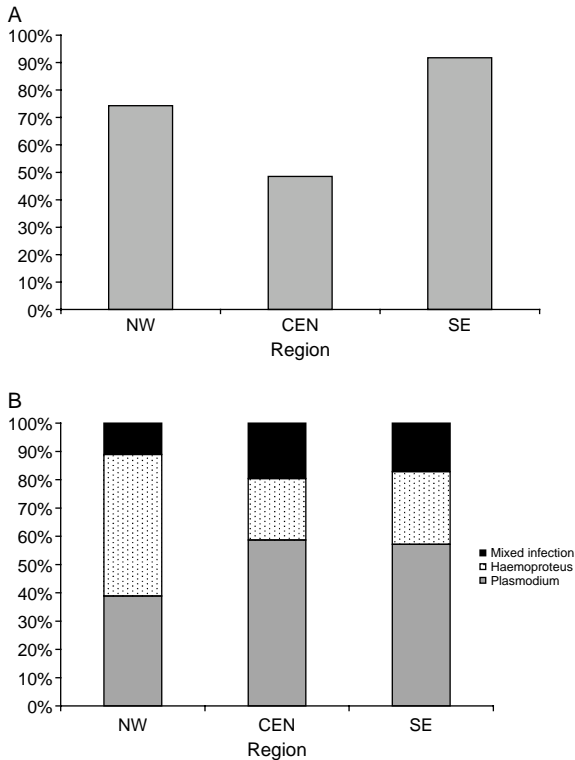| Site	n (birds sampled)	Overall prevalence (proportion infected)	*Plasmodium* infections (proportion infected)	*Haemoproteus* infections (proportion infected)	Mixed lineage infections (proportion infected)	Undetermined lineage infections (proportion infected)
NW	35	26 (0.74)	7 (0.27)	9 (0.35)	2 (0.08)	8 (0.30)
CEN	169	82 (0.49)	29 (0.35)	12 (0.15)	10 (0.12)	31 (0.38)
SE	73	67 (0.92)	20 (0.30)	9 (0.13)	6 (0.09)	32 (0.48)

Fig. 2. These histograms represent the total prevalence of haematozoan infection in the three regions of sampled red-billed quelea. A: Overall prevalence was significantly lower in the central region than either northwest or the southeast regions ($\chi^2 = 43.14$, df = 2, P = <0.001). B: Prevalence of differing types of haematozoan infection (when identified) was not significantly different between regions.

than that of the other two regions, its diversity was slightly higher and the confidence intervals do not overlap.

Correlation of plumage colour with infection status

The plumage scores of the sampled male quelea (n = 110) varied greatly. Belly hue ranged from reddish (H = 10) to nearly pure yellow (H = 59.75), with an average hue score of 42.83 (SE = 0.77). Throat hue ranged from brownish (H = 36.5) to bright red (H = 0.5) with an average hue score of 16.56 (SE = 0.98). The mask ranged from black (corrected K = 100%) to almost white (corrected K = 0.31%), with an average mask score of 70.72% (SE = 3.06). These scores were transformed to better approximate a normal distribution. The mask K scores were arcsine square-root transformed, the belly hue scores were square-root transformed and the throat hue scores were transformed

using the natural log. Normality was assessed using histograms with fitted normality curves.

There were no significant correlations between any of the three colour scores in the sample. Of the 110 males, 96 were infected with a haematozoan: i.e., prevalence was 87.3%, of which 33.33% were infected with a *Plasmodium* lineage, 15.63% were infected with *Haemoproteus*, 10.42% harboured a mixed infection of more than one lineage, and 40.63% could not have their lineage identified to genus. T-tests of the differences in mean colour scores for each plumage area between infected and non-infected males were all non-significant. A one-way anova of the differences of mean colour scores for the three plumage areas between males infected with *Plasmodium*, *Haemoproteus*, or a mixed infection was also non-significant. Finally, splitting the males up by regions (SE n = 68 (61 infected), CEN n = 14 (13 infected), and NW n = 28 (22 infected)) produced non-significant two-way anova test results for each of the three colour scores by infection status, region and the interaction between these two factors. There was, however, a significant difference in belly colour across the three regions (multivariate general linear model: df = 2, F = 4.365, P = 0.015). The observed power of most of these tests was low, ranging between 0.052 and 0.393, with the exception of the observed power of region predicting belly colour (obs. power = 0.744). Belly hue is significantly different between regions, and hue scores are higher in the south-east region, indicating more yellow-hued bellies on males in this area (one-way anova: df = 2, F = 10.171, P < 0.001).

Discussion

Our analysis of the distribution of haematozoan parasite assemblages of the red-billed quelea reflects previous analyses of the population structure and plumage characteristics of their hosts by Dallimer et al. (2003) and Dale (2000). This was apparent at all levels: fine-scale analysis of individual hosts showed no correlation between male plumage colouration and parasite presence, and broad-scale population-level analysis indicated there was no geographic structuring of parasite populations in a host that had similarly shown no phylogeographic structuring.

There was a significant difference in overall prevalence between the regions. The centre of the postulated migratory divide showed significantly lower prevalence of haematozoa infection than either the north-west or south-east regions. Although the vectors of avian haematozoa are poorly known, generally *Plasmodium* is thought to be transmitted by mosquitoes (Culicidae; Valkiunas 2005), and *Haemoproteus* is thought to be transmitted by biting flies (Hippoboscidae) and midges
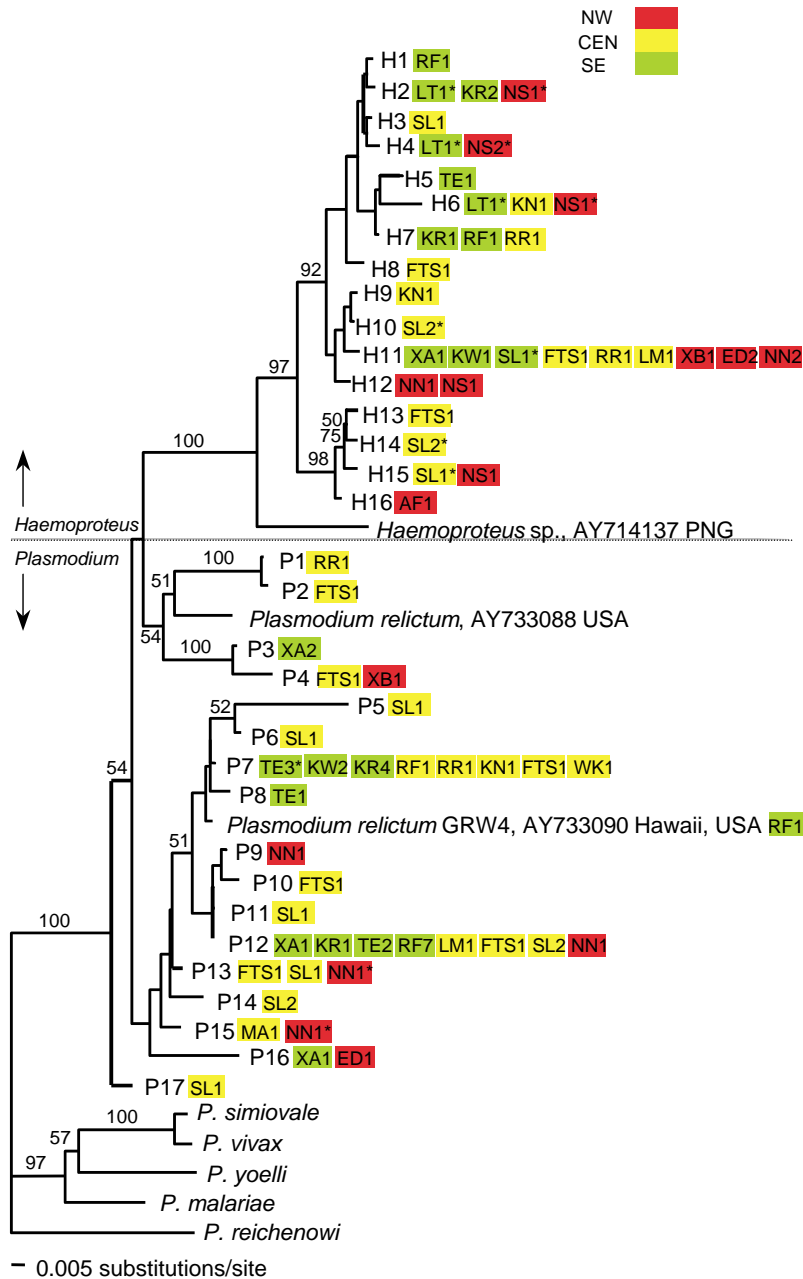
Fig. 3. N-J tree created with a heuristic search method and a Kimura 2-parameter evolutionary model, rooted with mammalian *Plasmodium* outgroups. Bootstrap (PAUP* N-J search) values ≈/ >50% are included on relevant branches. Numbers following haplotype names refer to the number of individuals in which haplotypes were detected, and each one is colour coded to match the region the individual was sampled from, red=northwest, yellow=central, and green=southeast. Note that the individuals that are labeled *Plasmodium relictum* are in different parts of the tree and likely represent distinct species (see Beadell et al. 2006).

(Ceratopogonidae; Atkinson et al. 1988, Martínez-Abraín et al. 2002). The rainfall patterns in these three regions, where the north-west and the south-east have recently received rain fronts while the central remained dry, may affect the population size of vectors, and lead to differences in prevalence of haematozoa in the hosts.

There was no difference in the prevalence of the different types of haematozoa infection across the three regions (Fig. 2). This may indicate that the transmission of all types of haematozoa is equally likely in all regions, or that there is no difference in vector assemblages in each region.

There was also no apparent phylogeographic structure in the haematozoa assemblages of the red-billed quelea in southern Africa. This reflects the lack of genetic population structuring in their hosts found by Dallimer et al. (2003), who suggested that the migratory divide does not split the population and that quelea interbreed freely across the whole of the subcontinent. The lack of host population structure may mean that the entire haematozoan population is panmictic and that the migratory divide does not split the parasite population. Alternatively, it may mean that the parasite vectors are not localized or that the parasites are transmitted when the quelea move through discrete populations of vectors from the central region outwards.

At the local scale of parasite distribution among individual hosts, there was no indication of a relationship between male plumage colouration and parasite infection status. This is consistent with the findings of Dale (2000), who suggested that the elaborate breeding plumage produced in the male is primarily for individual identification. The nesting colonies of breeding quelea are vast and densely packed, and individual recognition of males ensures that aggression is directed more towards distant neighbours (Crook 1960). However, our finding is contrary to that of other researchers who detected a correlation between haematozoan infection, carotenoid colouration and sexual selection in many organisms including fish and birds (reviewed in Olson and Owens 1998). The males analyzed in this study had a very high prevalence of infection. It may be preferable to test samples of equal numbers of infected and non-infected birds to see if there is any effect on plumage colouration. If the effect of parasite infection on plumage coloration were strong, then it should have been obvious in our results. The post-hoc power analyses of the two-way anova between region, infection and male plumage colour scores indicate that more equal sample sizes would be preferred. As we were limited to a sub-sample of birds from a broader study for which we had both post-mortem photographs and a blood or tissue sample, restricting our sample sizes to equal numbers of infected and non-infected breeding males would not have yielded sample sizes capable of generating any statistical power.

Our findings indicate that parasite population structure and distribution may not be affected at the individual host scale in this sample, or at the very least, that they are not reflected in plumage colouration. A lack of colourful breeding plumage may result in sexual selection against parasitized males, where the females avoid them to prevent mating, infection, or both. This would potentially lead to a change in the distribution of the parasites, which does not appear to be the case in this study.

Although our methodology did not discover any host structure that had gone undetected by other means of measuring host phylogeography, it did uncover a wealth of previously unknown lineages of avian haematozoa. It also detected a cryptic lineage with a global distribution, GRW4, which is of great concern to conservationists (Beadell et al. 2006). Given their often-cryptic nature, this many haematozoan lineages could not have been detected using standard microscopy techniques. While our molecular screening and identification protocol gives no information on intensity of infection, it relies less on observer skill and experience than traditional microscopy techniques. Furthermore, because parasite DNA can be amplified from samples with extremely low parasitaemia (Beadell and Fleischer 2005), our techniques yield a higher and probably more realistic estimation of prevalence. Given the widespread availability of genetic techniques, molecular approaches to identify and study haematozoan parasites should be adopted routinely and used in conjunction with microscopy-based morphological methods whenever feasible.

We detected the greatest number of haemoprotozoan lineages (n = 34 in 89 total sequences) ever discovered in a single host species, and Chao2 estimates indicate that there may be more to be discovered. However, other studies analysing large samples of a single host species recovered fewer lineages from populations showing similar prevalence of infection. Waldenström et al. (2002) uncovered 6 lineages of *Plasmodium* and *Haemoproteus* from a sample of 98 sedge warblers *Acrocephalus schoenobaenus* with a 33.7% overall prevalence of infection. Ricklefs et al. (2005) found 14 lineages from 29 sequences in 119 red-eyed vireos *Vireo olivaceus* with an infection prevalence of 52.9%. Ishtiaq et al. (2006) were able to detect 26 lineages in 93 sequences from a total screening population of 393 common mynas *Acriotheres tristis* with a prevalence of infection of 51%. Fallon et al. (2006) discovered 12 lineages from 189 sequences in 1083 black-throated blue warblers *Dendroica caerulescens*, with an overall infection prevalence of 21.8%. Pagenkopp et al. (in press) recovered 16 lineages in 210 sequences from 552 common yellowthroats *Geothlypis trichas* with an overall prevalence of 52.7%. The high diversity of haematozoan parasites in the red-billed quelea is an interesting aspect of this agricultural pest species. Whether this diversity is due to their abundance, their constant long-distance migratory movements, their immunocompetent ability, or a combination of factors remains to be discovered. Any link between these factors and the success of red-billed quelea in anthropogenic landscapes would be greatly informative to agencies charged with controlling their numbers.

References

Atkinson, C. T., Forrester, D. J. and Greiner, E. C. 1988. Epizootiology of *Haemoproteus meleagridis* (Protozoa: Haemosporina) in Florida: seasonal transmission and vector abundance. – J. Med. Entomol. 25: 45–51.

Beadell, J. S. and Fleischer, R. C. 2005. A restriction enzyme-based assay to distinguish between avian Hemosporidians. – J. Parasitol. 91: 683–685.

Beadell, J. S., Gering, E., Austin, J., Dumbacher, J. P., Peirce, M. A., Pratt, T. K., Atkinson, C. T. and Fleischer, R. C. 2004. Prevalence and differential host-specificity of two avian blood parasite genera in the Australo-Papuan region. – Mol. Ecol. 13: 3829–3844.

Beadell, J. S., Ishtiaq, F., Covas, R., Melo, M., Warren, B. H., Atkinson, C. T., Bensch, S., Graves, G. R., Jhala, Y. V., Peirce, M. A., Rahmani, A. R., Fonseca, D. M. and Fleischer, R. C. 2006. Global phylogeographic limits of Hawaii's avian malaria. – Proc. R. Soc. B 273: 2935–2944.

Biek, R., Drummond, A. J. and Poss, M. 2006. A virus reveals population structure and recent. demographic history of its carnivore host. – Science 311: 538–541.

Brawner, W. R., Hill, G. E. and Sundermann, C. A. 2000. Effects of Coccidial and Mycoplasmal infections on carotenoid-based plumage pigmentation in male house finches. – Auk 117: 952–963.

Colwell, R. K. 2005. EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. User's Guide and application published at: http://purl.oclc.org/estimates.

Criscione, C. D., Poulin, R. and Blouin, M. S. 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. – Mol. Ecol. 14: 2247–2257.

Crook, J. H. 1960. Studies on the social behaviour of *Quelea q. quelea* (Linn.) in French West Africa. – Behaviour 16: 1–55.

Dale, J. 2000. Ornamental plumage does not signal male quality in red-billed queleas. – Proc. R. Soc. B 267: 2143–2149.

Dale, J., Lank, D. B. and Reeve, H. K. 2001. Signalling individual identity versus quality: a model and case studies with ruffs, queleas and house finches. – Am. Nat. 158: 75–86.

Dallimer, M. and Jones, P. J. 2002. Migration orientation behaviour of the red-billed quelea *Quelea quelea*. – J. Avian Biol. 33: 89–94.

Dallimer, M., Jones, P. J., Pemberton, J. M. and Cheke, R. A. 2003. Lack of genetic and plumage differentiation in the red-billed quelea *Quelea quelea* across a migratory divide in southern Africa. – Mol. Ecol. 12: 345–353.

Dumbacher, J. P., Pratt, T. K. and Fleischer, R. C. 2003. Phylogeny of the owlet-nightjars (Aves: Aegothelidae) based on mitochondrial DNA sequence. – Mol. Phylogenet. Evol. 29: 540–549.

Elliot, C. C. H. 1989. The pest status of the quelea. In: Quelea quelea Africa's Bird Pest (eds. R. L. Bruggers and C. C. H. Elliott). – Oxford University Press, Oxford, pp. 17–34.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. – Biol. J. Linn. Soc. 41: 315–352.

Fallon, S. M., Fleischer, R. C. and Graves, G. R. 2006. Parasites as geographic markers in migratory birds? – Proc. R Soc. B Letters 2: 213–216.

Fleischer, R. C., Olson, S., James, H. F. and Cooper, A. C. 2000. The identity of the extinct Hawaiian eagle (*Haliaeetus*) as determined by mitochondrial DNA sequence. – Auk 117: 1051–1056.

Hamilton, W. D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites? – Science 218: 384–387.

Hill, G. E. 1998. An easy, inexpensive means to quantify plumage coloration. – J. Field Ornithol. 69: 353–510.

Houde, A. E. and Torio, A. J. 1992. Effect of parasitic infection on male colour pattern and female choice in guppies. – Behav. Ecol. 3: 346–351.

Hõrak, P., Ots, I., Vellau, H., Spottiswoode, C. and Møller, A. P. 2001. Carotenoid-based plumage colouration reflects hemoparasite infection and local survival in breeding great tits. – Oecologia 126: 166–173.

Ishtiaq, F., Beadell, J. S., Baker, A. J., Rahmani, A. R., Jhala, Y. V. and Fleischer, R. C. 2006. Prevalence and evolutionary relationships of haematozoan parasites in native versus introduced populations of Common myna *Acridotheres tristis*. – Proc. R. Soc. B 273: 587–594.

Jarvi, S. I., Schultz, J. S. and Atkinson, C. T. 2002. PCR diagnostics underestimate the prevalence of avian malaria (*Plasmodium relictum*) in experimentally infected passerines. – J. Parasitol. 88: 153–158.

Jones, P. J. 1989a. General aspects of quelea migrations. – In: eds Bruggers, R. L. and Elliott, C. C. H. *Quelea quelea* Africa's Bird Pest. Oxford University Press, Oxford, pp. 102–112.

Jones, P. J. 1989b. Distribution, populations, and migration patterns of quelea in southern Africa. – In: eds Bruggers, R. L. and Elliott, C. C. H. *Quelea quelea* Africa's Bird Pest. Oxford University Press, Oxford, pp. 132–143.

Maddison, D. R. and Maddison, W. P. 2000. MacClade 4: analysis of phylogeny and character evolution. Version 4.0, Sinauer Associates, Sunderland.

Martínez-Abraín, A., Merino, S., Oro, D. and Esparza, B. 2002. Prevalence of blood parasites in two western-Mediterranean local populations of the yellow-legged gull *Larus achinnans michahellis*. – Ornis Fennica. 79: 34–40.

McGraw, K. J. and Ardia, D. R. 2003. Carotenoids, immunocompetence, and the information content of sexual colours: an experimental test. – Am. Nat. 162: 704–712.

Moss, G. P. and Weeden, B. C. L. 1976. Chemistry of carotenoids, In: ed. Goodwin, T. W. Chemistry and biochemistry of plant pigments. – Academic Press, New York, New York, pp. 149-224.

Møller, A. P., Biard, C., Blount, J., Houston, D. C., Ninni, P., Saino, N. and Surai, P. F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? – Poult. Avian Biol. Rev. 11: 137–159.

Olson, V. A. and Owens, P. E. 1998. Costly sexual signals: are carotenoids rare, risky or required? – Trends Ecol. Evol. 13: 510–514.

Pagenkopp, K., Klicka, J., Durrant, K. L. and Fleischer, R. C. (In press) Geographic variation in malarial parasite lineages in the common yellowthroat (*Geothlypis trichas*), Cons. Genet.

Perkins, S. L. and Schall, J. J. 2002. A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. – J. Parasitol. 88: 972–978.

Ricklefs, R. E. and Fallon, S. M. 2002. Diversification and host switching in avian malaria parasites. – Proc. R. Soc. B 269: 885–892.

Ricklefs, R. E., Swanson, B. L., Fallon, S. M., Martínez-Abraín, A., Scheuerlein, A., Gray, J. and Latta, S. C. 2005. Community relationships of avian malaria parasites in southern Missouri. – Ecol. Monogr. 75: 543–559.

Saks, L., Ots, I. and Hõrak, P. 2003. Carotenoid-based plumage colouration of male greenfinches reflects health and immunocompetence. – Oecologia 134: 301–307.

Swofford, D. L. 1999. PAUP*: phylogenetic analysis using parsimony (*and other methods), Sinauer Associates, Sunderland.

Valkiunas, G. 2005. Avian malaria parasites and other haemosporidia. – CRC Press, Washington DC.

Waldenström, J., Bensch, S., Kiboi, S., Hasselquist, D. and Ottosson, U. 2002. Cross-species infection of blood parasites between resident and migratory songbirds in Africa. – Mol. Ecol. 11: 1545–1554.

Ward, P. 1966. Distribution, systematics, and polymorphism of the African weaver-bird (*Quelea quelea*). – Ibis 108: 34–40.

Ward, P. 1971. The migration patterns of *Quelea quelea*. Africa. – Ibis 113: 275–297.