Trans-oceanic host dispersal explains high seabird tick diversity on Cape Verde islands

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Parasites represent ideal models for unravelling biogeographic patterns and mechanisms of diversification on islands. Both host-mediated dispersal and within-island adaptation can shape parasite island assemblages. In this study, we examined patterns of genetic diversity and structure of Ornithodoros seabird ticks within the Cape Verde Archipelago in relation to their global phylogeography. Contrary to expectations, ticks from multiple, geographically distant clades mixed within the archipelago. Trans-oceanic colonization via host movements probably explains high local tick diversity, contrasting with previous research that suggests little large-scale dispersal in these birds. Although host specificity was not obvious at a global scale, host-associated genetic structure was found within Cape Verde colonies, indicating that post-colonization adaptation to specific hosts probably occurs. These results highlight the role of host metapopulation dynamics in the evolutionary ecology and epidemiology of avian parasites and pathogens.

Keywords: colonization; ectoparasite evolution; island biogeography; host specificity; local adaptation

1. INTRODUCTION
Islands represent hotspots of biodiversity and natural laboratories for studying evolution [1]. Yet, evolutionary studies on parasites of islands have lagged behind those on free-living organisms, and we know little about how parasites colonize, spread and adapt to these highly remote ecosystems (but see [2–4]). The main way in which parasites may reach islands is by tracking their original host. After island colonization, alien parasites may remain restricted to their original host and diverge from the source population via drift. They may also diverge locally after arrival by adapting to the new host community, i.e. host-associated adaptation [2]. The relative contribution of island colonization events and in situ diversification to local patterns of parasite diversity have been investigated for some avian blood parasites [5,6], but this question has received only limited attention for avian ectoparasites (but see [7]).

Seabird ectoparasite systems represent ideal models to investigate the processes and patterns of parasite diversification on oceanic islands. Seabirds are widely distributed, display colonial breeding in discrete island populations and tend to exhibit strong natal philopatry and interannual fidelity to breeding sites [8]. These repeated and long-term interactions between host and parasite should favour processes like local habitat adaptation and host specialization. However, seabirds can travel enormous distances and often breed in interspecific colonies, characteristics that may promote among-island dispersal and host switching in their parasites.

Soft ticks (Argasidae) of the Ornithodoros capensis species complex currently include eight morphospecies [9,10] that parasitize tropical and temperate seabirds of at least 12 families across the Pacific, Atlantic and Indian Oceans [8]. These ticks can be abundant in seabird colonies and can have direct negative effects on their hosts [11]. They can also harbour and transmit numerous disease agents [8], some of specific medical and veterinary concern [12].

Here, we examine the genetic diversity and structure of O. capensis ticks from Cape Verde to investigate the proximate determinants of tick biodiversity. Using a phylogeographic approach, we test whether standing parasite diversity has mainly resulted from within-island diversification or alternatively from repeated colonizations via seabird dispersal.

2. MATERIAL AND METHODS

(a) Tick sampling
Ticks were collected on hosts or from host nesting materials and preserved in 70 per cent ethanol. Population genetic analyses included 77 ticks from four seabird species (Cape Verde shearwaters, Calonectris edwardsii; brown boobies, Sula leucogaster; red-billed tropicbirds,Phaethon aethereus; and Cape Verde little shearwaters, Puffinus badius) and two islets within the Cape Verde Archipelago (Raso and Curral Velho; figure 1a). To place this diversity into a global context, we analysed 60 additional ticks collected from a variety of seabird species and geographical locations across the worldwide distribution of the complex (see electronic supplementary material, table S1).

(b) DNA isolation and PCR amplification
Each tick was washed in three sterile-water baths, air-dried and collected in a sterile microtube. Samples were individually crushed with a pestle, and DNA extractions were performed using the DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). Mitochondrial 16S and nuclear 18S rRNA genes were PCR-amplified for each tick (see electronic supplementary material, table S2 for details). Amplification products were sequenced in both directions at Beckman Coulter Genomics (France; GenBank accession nos. JQ824295–JQ824368).

(c) Genetic and statistical analyses
Chromatograms were checked and edited using GENEIOUS v. 5.3.6 (Biomatters Ltd Auckland, New Zealand). For the 18S nuclear locus, we checked for heterozygous individuals using the heterozygosity plugin in GENEIOUS. As no heterozygous positions were detected, nuclear haplotypes were used for subsequent analyses. Sequences were aligned for each gene independently by using MAFFT v. 6 (http://align.bmr.kyushu-u.ac.jp/mafft/server/), with default parameters.

Using ticks from Cape Verde only, we estimated statistical parsimony networks from the 16S and 18S sequences using TCS v. 1.21 [13], with default settings. For the worldwide analysis, we inferred phylogenies using Bayesian (BI) analyses on the global dataset comprising 137 ingroup taxa and one outgroup (O. moubata, GenBank accession no. L76355). BI analyses were run on each gene using MrBayes v. 3.2 [14], with identical haplotypes removed and using the best-fit model selected by JMODELTEST [15]. Two independent runs of 5 x 10^6 generations were performed, sampling trees every 1000 generations.

We examined host and island effects on patterns of genetic variation using a log-linear analysis (see the electronic supplementary material). For this analysis, we considered the four tick lineages from Cape Verde that parasitized the two principal seabird hosts found on both islands (C. edwardsii and S. leucogaster).

3. RESULTS
Within Cape Verde, we found 11 and five distinct haplotypes for the 16S and 18S genes, respectively (electronic supplementary material, figure S1). Statistical parsimony networks revealed high genetic divergence among ticks within the archipelago and suggested the local presence of several distinct lineages; 16S haplotypes grouped into five sub-networks (lineages I–V), whereas only three were found for 18S sequences. The majority of 16S clades were concordant with haplotype groups obtained for the 18S, with two exceptions (i.e. lineages II and IV).

There was no clear geographical segregation of tick lineages between islands in Cape Verde, but prevalence varied significantly among hosts: lineages I and V were more frequently associated with C. edwardsii, whereas lineages II and III mainly occurred on S. leucogaster (figure 1b). The log-linear analysis indicated a strong tick lineage–host–island interaction (table 1). The two-way comparisons revealed that this was mainly due to seabird host effects, both on the lineage distributions and the occurrence of ticks between islands. An island effect only became marginally significant after accounting for host effects (table 1).

The worldwide phylogenetic analysis of the individual genes (figure 2; electronic supplementary material, figure S2) resulted in similar topologies and revealed several divergent and well-supported clades. On these trees, Cape Verde lineages do not form a monophyletic group within the O. capensis complex, but rather are associated with ticks from distinct and widespread geographical regions (figure 2).

4. DISCUSSION
Seabird ticks from the Cape Verde archipelago fall into five distinct phylogenetic clades (coloured in figure 2) that include ticks from wide-ranging localities, i.e. South America, North Atlantic and Indian Oceans. This high diversity in Cape Verde is unexpected according to the classical hypotheses of parasite species richness on islands (i.e. parasite diversity is expected to be low and spatially restricted [16]). While sympatric host race formation and local adaptation may be common in ticks [7,17], our phylogenetic analyses indicate that the primary factor responsible for standing diversity within Cape Verde is multiple independent colonization events. A similar scenario of multiple colonizations and intra-island diversification has been recently reported for avian blood-parasites [6], but at a smaller spatial scale. The pattern of large-scale tick dispersal we observe here contrasts with the general notion that colonial seabirds are highly faithful to their breeding colonies and with genetic studies that indicate strong patterns of phylogeographic structure among ocean basins [18,19]. Inter-island dispersal in the absence of gene flow may occur in seabirds [20], for example, via immature and/or non-breeding seabirds, known to move over broad spatial scales [21]. Nonetheless, given the life history of soft ticks, we still expect successful tick dispersal to be a relatively rare event.

Within Cape Verde, tick lineages were not equally distributed among seabird host species on the two islands, resulting in specific tick–seabird host associations at a local scale and contrasting with an apparent lack of host-specificity at the worldwide scale (figure 2). Here, the use of low-resolution genetic markers and an incomplete coverage in the global sampling may limit our ability to identify specific tick–host associations, but taken together with patterns found at a local scale, our results suggest that host switching followed by adaptation may be common in this system. Indeed, although our
sampling is relatively limited for inferring island effects, our modelling suggests that geographical location only starts to become important after controlling for host effects. Both the stability of local host populations and an increase in antagonistic selection pressures at small scales could be responsible for such patterns [22], but will require population level analyses in other island systems to fully understand the microevolutionary processes at work.

5. CONCLUDING REMARKS

The biodiversity of parasites on island ecosystems and the factors that affect it are poorly understood. The high genetic diversity we found in *O. capensis* ticks within the Cape Verde Archipelago, as well as the fact that these coexisting lineages also occur on different host species in distant geographical locations, suggests that this diversity is largely a result of repeated colonization events. This study demonstrates the unappreciated frequency at which seabirds may disperse parasites over extreme spatial scales and, in doing so, their potential to spread any disease agents that they may carry. In this way, island systems like the Cape Verde Archipelago may represent particular biodiversity hotspots for avian parasites and pathogens, even more so than for their vertebrate hosts.

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