Evidence for multiple founding lineages and genetic admixture in the evolution of species within an oceanic island weevil (Coleoptera, Curculionidae) super-radiation.

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ABSTRACT

Aim To infer colonisation and speciation history for a closely related complex of nine species within the enigmatic Canary Island *Laparocerus* weevil radiation of 128 species. Using molecular dating and the spatial and temporal context that islands provide, we evaluate the possible explanations of incomplete lineage sorting and gene flow for the origin of shared genetic variation among species from different islands.

Location Canary Islands (Gran Canaria, Tenerife, La Palma and El Hierro).

Methods Phylogenetic analyses of mitochondrial (COII) and nuclear (ITS2) sequence data and molecular dating techniques were used to infer the origin of the group in the archipelago and their history of colonisation and differentiation.

Results Gran Canaria appeared to be the geographic origin of the complex. An unexpected result was that mtDNA revealed each of the single species on La Palma and El Hierro to be the product of more than one colonisation event from more than one source island. In both cases nuclear ITS2 data revealed these multiple colonisations to have been followed by admixture.

Main conclusions The two gene trees present very different topologies, with a rather simple colonisation history required to explain the pattern of nuclear gene relationships, while the mtDNA gene tree implicates a much more complex history of colonisation. Explanations of incomplete lineage sorting are ruled out and a history of colonisation and speciation for the *L. tessellatus* complex involving genetic admixture is inferred.

Keywords
Canary Islands, colonisation, diversification, *Laparocerus*, lineage sorting,

Macaronesia, speciation
INTRODUCTION

Charles Darwin’s stop in the Galapagos Islands during his five-year voyage on the HMS Beagle revealed to him that islands are an important source of evidence for evolution. Since Darwin’s time, islands have become not just a source of evidence, but theatres for investigating and understanding mechanistic explanations for evolution itself. This research effort has often focussed on particular islands and archipelagos, reflecting the fact that the evolutionary proliferation of biodiversity has progressed much farther on some islands than on others (Losos & Ricklefs, 2009). Interestingly, this proliferation often appears to be disproportionately distributed across co-occurring evolutionarily related taxa, with diversification having been much more extensive within some lineages than others. For example, among the 88 genera of weevil (Coleoptera, Curculionidae) occurring naturally within the Canary Islands, there is an average of only three species per genus (Oromí et al., 2010). However 128 species, more than 1/3 of all the native weevil species in the Canary Islands, belong to a single genus, Laparocerus Shonherr, 1834 (A. Machado, in prep.). This raises an important question, why do some lineages diversify so extensively on islands, while others do not?

The addition of molecular phylogenetic techniques to the evolutionary biologist’s toolkit has seen renewed focus on the study of evolution within oceanic islands over the last two decades, enabling researchers to address why a given lineage may have speciated more extensively in some archipelagos compared to others. However, the extent to which molecular phylogenetic analyses have gone beyond describing pattern
to inferring process has been limited, with most focusing on defining relationships among species, and inferring the timing of speciation events (e.g. Emerson et al., 2000; Emerson & Oromí, 2005; Amorim et al., 2012). Such analyses can help to elucidate the role of geography and the relative importance of within island and between island speciation. However, by typically sampling only a few individuals within a species, phylogenetic sampling does not capture diversification processes occurring below the species level.

Population-level studies that incorporate the sampling of individuals from across their range for the reconstruction of gene genealogies may help to reveal the extent to which natural selection or alternative mechanisms offer explanations of evolutionary change. Such studies have frequently revealed substantial genetic structuring within species within islands, and evolutionary processes underpinning diversification. The repeated convergent selection for, and evolutionary origins of, cave dwelling *Palmorchestia hypogaea* amphipod populations of the Canary Island of La Palma would have been all but missed with phylogenetic sampling (Villacorta et al., 2008), as would have been conclusions of ancestral and derived ecological associations and niche shifts in the *Nesotes* beetles of Gran Canaria (Rees et al., 2001). A revealing example of the combined power of geographic sampling with both mitochondrial and nuclear genomic sampling comes from Jordal et al.’s (2006) study of the sympatric and closely related *Aphanarthrum* weevil species *A. subglabrum* and *A. glabrum* on the island of La Palma in the Canary Islands. Representative geographic sampling of these two species and other taxa from the *A. glabrum* complex on other islands, combined with the analysis of mitochondrial and nuclear loci, revealed the combined
roles of geography (allopatric isolation and subsequent secondary contact) and species
interactions (hybridisation and reinforcement) in driving diversification.

With 128 described species from the Canary Islands and 34 described species from
the Madeira archipelago, the genus *Laparocerus* stands out as an evolutionary enigma.
It is estimated that there may be as many as 200 species distributed in the Canary and
Madeira archipelagos, with single species occurring in West Morocco, and the
Salvages Islands (Machado, 2011). It is the most species rich of all animal and plant
genera within the Canary Islands (Arechavaleta *et al.*, 2010). Species richness within
the *Laparocerus* is eight times higher than that of the next most species rich weevil
genus, *Acalles*, represented by 16 species (Oromí *et al.*, 2010), but there is little
understanding of what evolutionary processes may underpin the speciation of this
group. All *Laparocerus* species are flightless and most are oligophages that climb
vegetation to feed upon leaves, while a few species dwell in the leaf litter or are
adapted to the underground environment (Machado, 2003).

The extensive diversity within *Laparocerus* has for many years both intrigued and
confounded biologists, but recent efforts have partitioned this diversity into
taxonomic units (e.g. Machado, 2006; 2009), and species complexes within this
diversity have been delineated with both mtDNA and nuclear sequence data
(Machado *et al.*, 2008; A. Machado, in prep.). As a first approach to understand why
*Laparocerus* has diversified so dramatically within Macaronesia, we have sampled
within the well-defined *Laparocerus tessellatus* species complex of nine species
across its distribution on four of the Canary Islands (Gran Canaria, Tenerife, La
Palma and El Hierro). Using sequence data from mitochondrial cytochrome c oxidase
subunit II gene (COII) and the nuclear DNA internal transcribed spacer 2 gene (ITS2), we investigated inter-island colonisation within the complex, and the distribution of genetic variation among species within the complex. Using the spatial and temporal context that islands provide, we evaluate the possible explanations of incomplete lineage sorting and gene flow for the origin of shared DNA sequences among species from different islands, and discuss the implications of this shared variation for the speciation process in *Laparocerus*.

### METHODS

**Sampling and laboratory procedures**

The *Laparocerus tessellatus* complex consists of nine species that comprise a monophyletic group based on separate mitochondrial [cytochrome c oxidase subunit II gene (COII), 16S ribosomal RNA gene (16S), and the 12S ribosomal RNA gene (12S)], and nuclear [28SrDNA] sequence data. Bayesian Inference phylogenetic analysis of 243 *Laparocerus* operational taxonomic units (OTUs), including almost all described species of *Laparocerus* in the Canary Islands, reveals the complex to be monophyletic for both mtDNA and nuclear analyses with posterior probabilities (PP) of 1 in both cases (A. Machado, unpublished data). All species are single island endemics, with five on Gran Canaria (*L. microphthalmus* Lindberg, 1950, *L. obsitus Wollaston, 1864, L. osorio* Machado, 2012, *L. tirajana* Machado, 2012, and *L. sp. aff. tirajana*), two on Tenerife (*L. tessellatus* Brullé, 1839 and *L. freyi* Uyttenboogaart, 1940) and one on each of La Palma (*L. sp. 1*) and El Hierro (*L. bimbache* Machado, 2011). Samples were collected from 37 sites from 1999 to 2011 (Fig 1). A total of 173
specimens were collected from 10 sites in Gran Canaria, 18 in Tenerife, six in La Palma and three in El Hierro. Site locations and number of individuals per site are detailed in Table 1. *Laparocerus vicinus* Lindberg, 1953 (Gran Canaria) was used as an outgroup, based on its close phylogenetic relationship to the *L. tessellatus* complex. It is one of several closely related species that form a monophyletic group (PP = 1) with the complex in an analysis of mtDNA COII, 16S, 12S and nuclear 28S sequence data (A. Machado, unpublished data). Upon collection, samples were stored in absolute ethanol at 4°C prior to species identification and DNA extraction.

Total genomic DNA was extracted from the head and prothorax using the DNeasy 96 well Blood and Tissue Extraction Kit (QIAGEN, West Sussex, UK) following the manufacturer’s instructions, with the single exception that the head and prothorax were not ground. After extraction, both head and prothorax were placed back in absolute ethanol with the remainder of the body and maintained at 4°C as vouchers within the collection of A. Machado. Part of the mitochondrial COII gene and the nuclear ITS2 gene were amplified and sequenced as detailed in Appendix S1 (see Supporting Information). Sequencing was performed with the forward primer only for COII and in both directions for the ITS2, due to heterozygosity and indel (insertion and deletion) variation.

**Sequence alignment, haplotype reconstruction, and sequence properties**

All sequences were processed and ambiguous base calls manually assessed with **GENEIOUS PRO 5.4.5** (Drummond *et al.*, 2010). COII sequences were aligned using **MAFFT 6.814** (Katoh *et al.*, 2002) and ITS2 consensus sequences were aligned using
FAST 1.15.7 (Bradley et al., 2009), as it outperformed MAFFT when dealing with ITS2 indel variation. Both alignments were then checked by eye. Haplotypes for ITS2 sequences that were multi-site heterozygotes for single nucleotide polymorphisms (SNPs) were resolved either by direct comparison to homozygous sequences or with PHASE 2.1.1 (Stephens et al., 2001; Stephens & Scheet, 2005). The web tool seqPHASE (Flot, 2010) was used to create PHASE input files and to interpret PHASE output files. Haplotype determination for out of phase sequence traces, caused by alleles with indel variation, was inferred from related homozygous sequences, or with the program PHASE (Stephens & Scheet, 2005). The web tool SEQPHASE (Flot, 2010) was used to create PHASE input files and to interpret PHASE output files. Haplotype determination for out of phase sequence traces, caused by alleles with indel variation, was inferred from related homozygous sequences, or with the program PHASE (Stephens & Scheet, 2005).

The total number of variable and parsimony informative sites, average and maximum pairwise genetic distances (both uncorrected and corrected) overall, within and between species were computed with MEGA 6.0 (Tamura et al., 2013). The entropy-based index as implemented in DAMBE 5.2.78 (Xia et al., 2003) was used to assess substitution saturation within the mtDNA and ITS2 genes and the RPD3 software (Martin et al., 2010) was applied to detect potential historical recombination events within the ITS2 nuclear gene.

**Evolutionary tree and haplotype network construction**

Bayesian inference (BI) phylogenetic analyses were performed for the mtDNA and nuclear gene sequence alignments separately, using the parallel version of MrBayes 3.2.1 (Ronquist, 2012). Eight analyses were run each for 10 million generations using 8 MCMC (Markov chain Monte Carlo) chains, discarding 25% of samples as burn-in. For both genes the general time reversible model of sequence evolution with a gamma
correction (GTR + G) was used without modelling invariant sites (I), as recommended by Ren et al., (2005), Siekmann et al., (2012) and Jia et al., (2014, and references therein), with priors set to the default values. Trees were rooted with *L. vicinus*. The parameter estimates were assessed for stationarity and convergence in TRACER v.1.5 (Rambaut & Drummond, 2009) with only estimated sample size (ESS) above 200 for all parameters being accepted. Trees were visualised in FIGTREE 1.3.1 (Rambaut, 2011). TCS v.1.21 (Clement, 2000) was employed to infer haplotype networks of the less divergent nuclear gene sequences using statistical parsimony (Templeton *et al*., 1992) with 95% confidence limit, and the software HAPSTAR (Teacher & Griffiths, 2011) was used to draw the network.

**Dating analysis**

When trying to identify the evolutionary processes that shape species formation, distinguishing between introgression and incomplete lineage sorting is generally difficult (Toews & Brelsford, 2012). However oceanic islands offer an ideal framework to unravel these processes, mainly due to the dynamics of speciation by founder events. In an insular setting, incomplete lineage sorting is of minor consequence within species derived from a limited number of founding individuals from a different island. Thus, gene flow among species that are derived from independent colonisation events can be evaluated in a background of limited or no incomplete lineage sorting (e.g. Jordal *et al*., 2006; Nietlisbach *et al*., 2013). To further evaluate the possibility of incomplete lineage sorting, we use the spatial context provided by islands and the temporal information provided by DNA sequences to infer the timing and direction of colonisation events that are needed to
explain shared genetic variation between species on different islands. In the absence of a calibrated rate for *Laparocerus*, we applied a general coleopteran COII mutation rate of 0.015 substitution/site/myr (Cicconardi *et al.*, 2010) with a restricted uniform distribution interval [0.0149, 0.0151]. Our primary interest is not the absolute timing of lineage colonisation, but the relative timing. Thus absolute age estimates are intended to provide temporal context, under the untested assumption that the *Laparocerus* COII mutation rate is consistent with the general coleopteran rate estimate of Cicconardi *et al.* (2010). We use a strict molecular clock, as this is preferred over a relaxed clock for intraspecific genetic variation (Barnett *et al.*, 2014).

The MrBayes output trees for the mtDNA gene partition were used as starting trees for the dating analyses with BEAST 1.7.3 (Drummond *et al.*, 2012). Analyses used a GTR+G substitution model with 4 gamma categories, a Yule tree prior, and nodes with posterior probability (PP) of 0.90 or higher constrained to be monophyletic.

To account for the possibility of either the extinction or non-sampling of molecular lineages, nodes representing both the earliest and the most recent possible lineage colonisation times were estimated, and the time intervals among colonising lineages compared. For a more detailed discussion of this approach see Emerson (2002), Figure 6. Input files were generated in BEAUTI 1.7.3 (Drummond *et al.*, 2012), and 15 runs of 100 million generations each, sampled every 1000 generations, were performed and combined, checking sampling, mixing and convergence to a stationary distribution with TRACER 1.5 (Rambaut & Drummond, 2009).

**RESULTS**
Mitochondrial COII gene

All but one of the 173 specimens were successfully amplified and sequenced for the mitochondrial COII gene, producing an alignment of 172 sequences of 633bp. Across the ingroup, 122 polymorphic sites (of which 96 were parsimony informative) and 76 unique alleles were identified. The average pairwise p-distance was 4% across all species with a maximum of 6.9%. Within species, average pairwise p-distances ranged from 0.7% (L. osorio) to 3.9% (Laparocerus sp. 1), and between species, average pairwise p-distances ranged from 1.7% (L. osorio and L. microphthalmus) to 5.2% (L. bimbache and L. tirajana) (Table 2).

Xia’s index for substitution saturation produced values of 0.015 (first and second codon positions) and 0.16 (third codon position) which were significantly lower than the critical value for symmetric topologies (0.69-0.79, P<0.001; 0.64-0.77, P<0.001, respectively), suggesting that sites have reached little saturation and sequences can be reliably used for phylogenetic reconstruction.

Nuclear ITS2 gene

All but two of the 173 sampled beetles were successfully amplified and sequenced for the ITS2 gene. Of the 170 beetles of the ingroup, 64 were homozygotes and 106 were heterozygotes. All but five heterozygotes were successfully resolved for indel and SNP variation (phase threshold > 85%). The five unphased samples were removed from the dataset. The number of SNPs per heterozygote ranged from 1 to 6. Indels ranged in length from 1-19 bp and only one individual presented more than a single
indel differing between its two alleles. The ITS2 alignment consisted of 330 sequences resulting in a final alignment of 411 bp after the removal of two variable poly-A regions, and a hyper-variable region of 35 bp that could not be aligned. Across the ingroup there were 48 polymorphic sites (of which 24 were parsimony informative) and 52 unique alleles were identified. The average pairwise p-distance was 1%, with a maximum of 3.4%. Within species, average pairwise p-distance ranged from 0.3% (L. tirajana) to 1.4% (L. sp. aff. tirajana), and between species, average pairwise p-distance ranged from 0.4 (Laparocerus sp. 1 and L. bimbache) to 2.5% (L. microphthalmus and L. tessellatus) (Table 3).

Xia’s index for substitution saturation, performed on all sites for the nuclear ITS2 dataset, produced values of 0.10-0.15 which were significantly lower than the critical values for symmetric topologies (0.69-0.79, P<0.001), suggesting that sites have reached little saturation and sequences can be reliably used for phylogenetic reconstruction. No signal of recombination was detected for the ITS2 gene for any of the methods used.

Phylogenetic analysis of mtDNA COII gene sequences

The bayesian tree of mtDNA sequence data reveals most of the morphologically described species within the L. tessellatus complex to be paraphyletic or polyphyletic for this gene (Fig 2). Four major groups are described by the mtDNA sequence data: two poorly supported but geographically distinct clades (1 and 2) and two well supported clades (3 and 4). Within the island of Gran Canaria, L. osorio is the only species clearly recovered as monophyletic (Posterior Probability, PP=0.98). The two
species from Tenerife, *L. freyi* and *L. tessellatus* are polyphyletic and share four mtDNA haplotypes (h15, h25, h29 and h76). *Laparocerus* sp. 1 from La Palma is polyphyletic, originating from three founding mtDNA lineages (uncorrected p-distance among lineages ranges from 3.2 to 6.3%). The first of these lineages (Lap-1) comprises clade 3 with sequences from Gran Canaria (PP=1). The second (Lap-2) and third (Lap-3) La Palma lineages comprise clade 4 with sequences from Tenerife and El Hierro (PP=1). Within clade 4, lineage Lap-2 forms a moderately supported clade with sequences from Tenerife (PP=0.86), while Lap-3 contains sequences from El Hierro and forms a highly supported clade with sequences from Tenerife (PP=0.95).

Similar to La Palma, mtDNA sequences from the El Hierro species *L. bimbache* are polyphyletic with two lineages of independent origin (uncorrected p-distance between lineages 5.3%). The first of these (Bim-1) forms a strongly supported clade (PP=1) with sequences from La Palma while the second lineage (Bim-2) is more closely related to sequences from Gran Canaria.

**Phylogenetic and network analyses of nuclear ITS2 sequences**

Within the bayesian ITS2 tree the earliest branching events are comprised solely of DNA sequences from Gran Canaria, with the remaining sequences from Gran Canaria forming a well supported clade (clade C, PP=1) with sequences from the remaining islands (Fig. 3). Only *L. microphthalmus* and *L. tirajana* from Gran Canaria are recovered as monophyletic. The Tenerife species *L. freyi* and *L. tessellatus* are polyphyletic and share six haplotypes (h6, h20, h21, h24, h29, and h28). For a more resolved understanding of haplotype relationships within the clade comprising sequences from all islands (clade C), they were used for a network analysis, with
closely related sequences from *L. osorio* (h48, h49 and h50) included as an outgroup to provide temporal information regarding derived and ancestral haplotypes (e.g. Zarza et al., 2008). A single network with no reticulations among haplotypes was recovered (Fig. 4) with haplotype h20 from Tenerife identified as the most recent common ancestor (mrca) haplotype within the clade. Haplotype h20 would appear to be either unsampled, or extinct on Gran Canaria, given that *L. sp. aff. tirajana* haplotypes (h42 and h43) are directly derived from it. Two haplotypes are shared between Tenerife and La Palma (h2 and h6), with one of these (h2) also being shared with El Hierro (Fig.4).

**Dating analysis**

The BEAST analysis of mtDNA COII sequences yielded an estimate of approximately 6.32 Ma [95% highest posterior density (HPD): 4.42-8.32 Ma] for the divergence of the *L. tessellatus* complex from *L. vicinus*, with initial diversification within the complex estimated at approximately 2.71 Ma [HPD 2.08-3.38]. These two absolute age estimates are tentative, as they are reliant on a general coleopteran mtDNA rate, and thus should be viewed as uncertain. Other node age estimates are used to compare earliest and most recent possible times for inter-island DNA among different sequence colonisation events (Fig. 2 and Table 4).

Lap-2 and Lap-3 are derived within lineage 4, a clade of mitochondrial diversity otherwise endemic to Tenerife (Fig. 2), indicative of La Palma having been colonised by two mitochondrial lineages from Tenerife. Lap-2 is estimated to have colonised between 0.21 Ma [0.06-0.4] (node D) and 0.07 Ma [0.02-0.16] (node E), and Lap-3
between 0.98 Ma [0.63-1.35] (node F) and 0.38 Ma [0.21-0.55] (node G). All remaining La Palma sequences occur together with Gran Canaria sequences in lineage 3. The absence of basal resolution within this lineage means that it is not possible to infer if La Palma sequences are derived from Gran Canaria, or visa versa. If La Palma sequences are derived from Gran Canaria, then this colonisation is estimated to have occurred approximately 0.96 Ma [0.66-1.28] (node C). The alternative scenario of a La Palma origin would mean that La Palma is estimated to have been colonised at least 0.96 Ma [0.66-1.28] from an undetermined source.

Bim-1 and Bim-2 are derived within lineages of sequences otherwise endemic to La Palma and Gran Canaria respectively, indicating a colonisation of El Hierro by a La Palma lineage between 0.29 Ma [0.15-0.45] (node H) and 0.12 Ma [0.02-0.23] (node I) and by a lineage from Gran Canaria between 0.4 Ma [0.14-0.71] (node J) and 0.08 Ma [0.015-0.19] (node K).

**DISCUSSION**

We investigated the history of diversification within the *L. tessellatus* species complex in the Canary Islands using a combination of sequence data from one mitochondrial and one nuclear gene. Both gene trees are consistent with the progression rule hypothesis (Funk & Wagner, 1995), where younger islands are colonised from older islands. However, the two gene trees present very different topologies, with a rather simple colonisation history required to explain the pattern of nuclear gene relationships, while the mtDNA gene tree implicates a much more complex history of colonisation. Using the geographic context of the islands
themselves, and relative temporal information from the gene trees, we were able to identify the geographic origin of the complex, and dismiss explanations of incomplete lineage sorting to reveal a history of colonisation and speciation involving genetic admixture.

*Geographic origin of the L. tessellatus complex*

The nuclear sequence data indicates the geographic origin of the complex to be Gran Canaria. The earliest branching events within the ITS2 tree are uniquely composed of individuals from Gran Canaria, with sequences from all other islands restricted to a single well-defined clade that also includes DNA sequences from Gran Canaria (clade C, Fig. 3). The complex relationships within the clade composed of sequences from all islands are best understood when viewed as a network (Fig. 4). All nuclear ITS2 DNA sequence variation sampled on the islands of Tenerife, La Palma and El Hierro is derived from a single ancestral sequence that is identified as haplotype h20 from Tenerife. Given the inferred Gran Canaria origin for the complex, the most parsimonious explanation for the absence of h20 on Gran Canaria is that it is either unsampled or extinct. The alternative explanation that h20 was never present on Gran Canaria would require h42 and h43 to have both colonised Gran Canaria from Tenerife, with these two haplotypes being unsampled or now extinct on Tenerife. This alternative explanation is less parsimonious as it requires two colonisation events (c.f. 1), two unsampled or extinct haplotypes (c.f. 1), and would implicate admixture for *L. tirajana*. Relationships among mtDNA sequences within the complex provide little evidence that can be used to infer the geographic origin of the group. However, a Bayesian Inference phylogenetic ana
lysis of 243 Laparocerus OTUs, including almost all described species of
Laparocerus in the Canary Islands, places the complex within a well-supported
mtDNA clade (PP=1) comprised of an additional 19 species endemic to Gran Canaria,
with only 3 non-Gran Canarian species (A. Machado, unpublished data), consistent
with a Gran Canarian origin for the complex.

La Palma and El Hierro – single species with multiple origins

The ITS2 sequence variation on La Palma and El Hierro can be explained by a single
source island in each case. ITS2 sequences from La Palma are consistent with
Tenerife as a source, while those of El Hierro can be explained by an origin from
either La Palma or Tenerife (Figs. 3 & 4). While more complex scenarios are also
possible (e.g. El Hierro colonised from both Tenerife and La Palma), a simple single
island origin is not rejected by the data. In contrast, mitochondrial relationships are in
sharp disagreement with a simple colonisation scenario. Laparocerus sp. 1 (La Palma)
is composed of multiple mitochondrial lineages, derived from either Tenerife (L. freyi
and L. tessellatus) or Gran Canaria (L. tirajana and L. sp. aff. tirajana) (Fig. 2).

Laparocerus bimbache (El Hierro) is composed of two mitochondrial lineages, one
derived from Laparocerus sp. 1 (La Palma), while the other is derived from within L.
microphthalmus (Gran Canaria) (Fig. 2). This pattern of mixed ancestry suggests
either: (i) species origin on La Palma and El Hierro involving genetic admixture from
multiple founding species (Fig. 5), or (ii) incomplete lineage sorting (Funk & Omland,
There is increasing evidence for genetic admixture following multiple founding events within oceanic island settings. Jordal et al. (2006) revealed historical gene flow among at least two founding populations of the *Aphanarthrum glabrum* species complex on the island of La Palma, followed by the evolution of reproductive isolation. Historical genomic admixture among species originating from different islands is invoked to explain discordant mito-nuclear tree topologies for a subspecies of the Galápagos mockingbird genus *Mimus* (Nietlisbach et al., 2013) and species within the cricket genus *Lapaula* in the Hawaiian islands (Shaw, 2002). In contrast, Garrick et al. (2014) describe extensive and ongoing introgression within the Galápagos giant tortoise species *Chelonoidis becki* on the island of Isabela involving two founding populations of *Chelonoidis darwini* from the island of Santiago. It has recently been documented within Darwin’s finches of the Galápagos archipelago that there has been extensive interspecific gene flow subsequent to independent colonisation events to the same island, with hybridisation giving rise to species of mixed ancestry (Lamichhaney et al., 2015).

We argue that the genetic data for the *Laparocerus tessellatus* complex species of La Palma and El Hierro is also a consequence of genetic admixture involving multiple founding species, rather than incomplete lineage sorting. Distinguishing between these two processes is typically a challenge, but the dynamics of speciation by founder events between islands enables us to exclude lineage sorting by evaluating three expectations from incomplete lineage sorting. We deal with each of these three expectations below in turn.
Expectation 1: All sequence variation shared among islands was present in the ancestral gene pool. This would require colonisation events between islands involving large numbers of founding individuals, such that a substantial amount of the standing ancestral genetic variation would be transferred. This is not consistent with the colonisation dynamics of flightless beetles (Ikeda et al., 2012; Vogler & Timmermans, 2012). Even if such a colonisation dynamic were to have occurred, it would then need to be followed by non-random extinction (or sampling) of genetic variation within both the source and founded islands. As an example, the following scenario would be necessary to explain patterns of genetic diversity on La Palma if Laparocerus sp. 1 was the result of a single colonisation event from either Gran Canaria or Tenerife. Extant sequence variation within lineage 4 (Fig. 2) prior to the colonisation event would have been present within the ancestral population of Gran Canaria. Additionally, sequence variation within lineage 3 (Fig. 2) would have been present within Tenerife. Reciprocal and extensive mtDNA lineage extinction on both islands would then be required to explain the absence of Tenerife sequences within lineage 3, and the absence of Gran Canaria sequences in lineage 4. We have formally evaluated incomplete lineage sorting as an explanation for non-shared genetic variation between Gran Canaria and Tenerife, which is clearly rejected (Appendix S2).

Expectation 2: If the species from La Palma and El Hierro are each the product of a single colonisation event, colonisation times of all molecular lineages shared between these and other islands should coincide. The relative time interval for the colonisation of a lineage to an island ranges from the lowest to the highest bound of the two time intervals (earliest and most recent possible) measured for each lineage, e.g. La Palma was colonised from one Tenerife lineage between 0.02-0.4 Ma (minimum and
maximum HPD values for nodes D and E, Fig. 2). There is substantial overlap in the
time intervals estimated for different lineages colonising La Palma and El Hierro, and
this is a consequence of the wide 95% posterior intervals estimated by the Bayesian
MCMC method when dealing with shallow genetic divergence (Brown & Yang,
2010). Despite this difficulty, results clearly reject the hypothesis of a single
colonisation for La Palma. MtDNA sequence diversity within Lap-2 is estimated to be
derived from a colonisation event no older than 0.4 Ma (node D, Fig. 2). In contrast,
mtDNA sequence diversity within Lap-1 is estimated to either be derived from a
colonisation event from Gran Canaria no younger than 0.66 Ma (node C, Fig. 2), or to
have originated much earlier than this date. Thus temporal information alone indicates
that La Palma was colonised at least two times.

Expectation 3: A signature of incomplete lineage sorting for nuclear gene is expected
to be more exaggerated than for the mitochondrial gene, due to its much bigger
effective population size (Ballard & Whitlock, 2004). In contrast to this expectation,
the signature of potential incomplete lineage sorting among islands presented by the
nuclear (Fig. 4) is much less than expected if the patterns of mtDNA relatedness are
due to incomplete lineage sorting. If we consider the shared mtDNA variation
observed between Gran Canaria and both La Palma and El Hierro to be the result of
incomplete lineage sorting, its absence within the more slowly evolving nuclear gene,
with its much larger effective population size becomes difficult to explain. It should
be noted that there are phenomena that can tend to increase the effective population
size of mtDNA relative to nuclear DNA, such as female biased sex ratios, or mating
systems that favour high variance in male reproductive success. While there is no
information on mating system, sex ratios estimated from 142 individuals of L.
tessellatus, 67 L. obsitus and 88 Laparocerus sp. 1 are 0.9, 1.0 and 0.8 respectively (A. Machado, unpublished data), indicate a slight, or no bias, within species.

Species boundaries in Tenerife

Most DNA sequences sampled within L. tessellatus were also found in L. freyi suggesting that L. tessellatus may be of recent origin from an L. freyi like ancestor. Of the five mitochondrial and eight nuclear sequences sampled from L. tessellatus, four and six are shared with L. freyi. While the two species are not distinct at the genetic level, there is an interesting genetic observation at two sampling sites where two divergent mtDNA lineages were sampled in sympatry. Both sites are represented by only one of the two species. This observation provides an opportunity to evaluate whether mtDNA divergence within Tenerife may be associated with cryptic species (see details in Appendix 2). While at site TF48 (L. freyi), the two mtDNA lineages show no evidence that they might represent biological species, at site TF45 (L. tessellatus), there is a strong nuclear signature of limited gene flow among individuals from these two mtDNA lineages. This complex genetic pattern may be indicative of differences in environmental backgrounds facilitating contact and gene exchange between divergent gene pools at one site, while enhancing isolation and limiting gene flow at another. A similar phenomenon has been reported for Towhee birds Pipilo maculatus and P. ocai in southern Mexico, which hybridize extensively in several sites, but coexist in sympatry with no evidence of gene flow in other sites (Sibley & Sibley, 1964). However, FIS values at both sites, as well as three further sites sampled for only a single mtDNA lineage, were all found to be significantly positive (Appendix 2), indicating that rather than species boundaries, apparent nuclear genetic
differentiation between divergent sympatric mtDNA lineages could more plausibly be a consequence of non-random mating within sampling sites. Further sampling will be required to understand what drives the structuring of genetic variation within sampling sites.

Conclusions

Species that are the product of genetic admixture among multiple colonising lineages within island archipelagos, as demonstrated here, have rarely been reported for animal taxa. However, this phenomena may not be unexpected, because island archipelagos present a geographic matrix where both geographic isolation and secondary contact are a function of colonisation dynamics and frequency in space and time (Emerson & Faria, 2014). High colonisation rates among islands will push populations toward panmixia, while low colonisation rates will facilitate divergence. At intermediate rates, where genomic divergence is promoted, but where barriers to gene flow may not have reached completion, the potential for admixture may arise. Indeed, genomic admixture following multiple founding events has been demonstrated within Galápagos giant tortoises (Garrick et al., 2014), and invoked to explain discordant mito-nuclear tree topologies for a subspecies of the Galápagos mockingbird genus *Mimus* (Nietlisbach et al., 2013). Our findings, like those of Nietlisbach et al. (2013) and Garrick et al. (2014) raise an interesting question – to what extent might admixture be a driver of diversification itself? The potential importance of genetic admixture as a driver of speciation is well recognised (Shaw, 2002; Mallet, 2007; Schwenk et al., 2008). In the context of island colonisation, founding events that involve only one or a few individuals will result in low genetic diversity within the
founding populations, which can only be recovered over a mutational evolutionary time-scale mutation. Genetic admixture provides a potential escape from reduced genetic variance via recombination among divergent genomes that may also facilitate adaptation within novel adaptive landscapes (Mallet, 2007; Emerson & Faria, 2014). Further work is needed to address this issue, and new techniques such as reduced genome sequencing and genotyping by sequencing (e.g. Mastretta-Yanes et al., 2014) should prove very useful in this respect.

Acknowledgements

We are grateful to the constructive comments of three anonymous referees, which helped to improve previous version of this manuscript. This study was supported by FCT-PTDC/BIA-BEC/104571/2008 granted to I.R.A and P.A.V.B, by Spanish MINECO grant CGL2013-42589-P awarded to B.C.E., and by the School of Biological Sciences, University of East Anglia. C.M.A.F was funded by a Brazilian Council for Scientific and Technological Development PhD studentship (CNPQ – 200439/2010-3).
References


**Supporting Information**

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

**Appendix S1** Table S1 and Details of PCR amplification and sequencing used in this study.

**Appendix S2** Simulation of stochastic lineage sorting and Analysis of species boundaries.

**Appendix S3** Full Bayesian phylogenetic trees of the mtDNA COII data set (Fig. S1) and ITS2 nuclear gene dataset (Fig. S2).

**Data Accessibility**

Mitochondrial DNA sequences and ITS2 nuclear sequences used in this study are available for download at GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under accession numbers ######

**Biosketch**

**Christiana M. A. Faria** is a PhD student at the Centre of Ecology, Evolution and Conservation, University of East Anglia. She is interested in applying molecular phylogenetic methods to studies of evolutionary ecology, biogeography, species adaptation and diversification processes.
Author contributions: C.M.A.F carried out molecular work and analysed the data; C.M.A.F and B.C.E. wrote the manuscript, and all authors discussed the data and commented on the manuscript; A.M. collected the samples; B.C.E. conceived and supervised the project.

Editor: Aristeidis Parmakelis
Table 1 - Details of sampling within the Canary Islands for species within the *Laparocerus tessellatus* complex. Sites coded according to Fig 1. Geographic coordinates are provided in Table S1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Site code</th>
<th>Site</th>
<th>n individuals</th>
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<td></td>
<td>LP2</td>
<td>La Palma: Llanada de Barlovento, 650 m</td>
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<tr>
<td></td>
<td>LP3</td>
<td>La Palma: s. El Paso, 870 m</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>LP4</td>
<td>La Palma: Breña Alta, Pared Vieja, 1350 m</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>LP6</td>
<td>La Palma: Mazo: Venijobre, 830 m</td>
<td>29</td>
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<td></td>
<td>LP7</td>
<td>La Palma: El Paso: Mña Don Mendo, 1075 m</td>
<td>3</td>
</tr>
<tr>
<td><em>L. bimbache</em> Machado, 2011</td>
<td>EH9</td>
<td>El Hierro: Monte Ajares, 600 m</td>
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</tr>
<tr>
<td></td>
<td>EH10</td>
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<td></td>
<td>EH11</td>
<td>El Hierro: Cruz de Isora, Infra Masilva, 1247 m</td>
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<td><em>L. freyi</em> Uyttenboogaart, 1940</td>
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<td>TF13</td>
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<td>TF14</td>
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<td>TF15</td>
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<td>TF21</td>
<td>Tenerife: s. Mña. Bermeja, 1600 m</td>
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<td></td>
<td>TF22</td>
<td>Tenerife: Güimar: Bco. del Agua, 700-800 m</td>
<td>9</td>
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<td></td>
<td>TF23</td>
<td>Tenerife: s. Icod El Alto, 1200 m</td>
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<td>Code</td>
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<td></td>
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<tr>
<td>------</td>
<td>----------------------</td>
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</tr>
<tr>
<td>TF24</td>
<td>Tenerife: Santa Úrsula: Bco. Bensa, 1463 m</td>
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<td>TF25</td>
<td>Tenerife: Tacoronte: FuenteFria, 1014 m</td>
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<td>Tenerife: Santa Úrsula: La Corujera, 600 m</td>
<td></td>
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<tr>
<td>TF28</td>
<td>Tenerife: El Portillo, 2000 m</td>
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<tr>
<td>TF48</td>
<td>Tenerife: Las Raices, 930 m</td>
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<td></td>
</tr>
<tr>
<td>TF49</td>
<td>Tenerife: Ifonche, 990 m</td>
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<td></td>
</tr>
<tr>
<td>TF43</td>
<td>Tenerife: Pista LasYedras, 740 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF45</td>
<td>Tenerife: Anaga: El Pijaral Km 4.5, 700 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF46</td>
<td>Tenerife: Anaga: Chinobre, 900 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF47</td>
<td>Tenerife: Anaga: Cruz del Carmen, 900 m</td>
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*L. tessellatus* Brullé, 1839

<table>
<thead>
<tr>
<th>Code</th>
<th>Location Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC32</td>
<td>Gran Canaria: Tamadaba NW, 1200 m</td>
</tr>
<tr>
<td>GC31</td>
<td>Gran Canaria: Valsendero: Bco. Oscuro, 900 m</td>
</tr>
</tbody>
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*L. microphthalmus* Lindberg, 1950

<table>
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<th>Code</th>
<th>Location Description</th>
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<td>Gran Canaria: San Bartolomé, Km 1, 940 m</td>
</tr>
<tr>
<td>GC36</td>
<td>Gran Canaria: San Bartolomé: Bco. Tirajana, 900 m</td>
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*L. obsitus* Wollaston, 1864

<table>
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<th>Code</th>
<th>Location Description</th>
</tr>
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<td>Gran Canaria: Bco. de los Cernícalos, 1400 m</td>
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<tr>
<td>GC37</td>
<td>Gran Canaria: Degollada de Osorio, 875 m</td>
</tr>
<tr>
<td>GC39</td>
<td>Gran Canaria: Cumbre: Roque Redondo, 1900 m</td>
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*L. tirajana* Machado, 2012

<table>
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<tr>
<td>GC37</td>
<td>Gran Canaria: Degollada de Osorio, 875 m</td>
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<tr>
<td>GC39</td>
<td>Gran Canaria: Cumbre: Roque Redondo, 1900 m</td>
</tr>
</tbody>
</table>

*L. sp. aff. tirajana*

<table>
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<th>Location Description</th>
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<tbody>
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<td>GC34</td>
<td>Gran Canaria: Bco. de los Cernícalos, 1400 m</td>
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<td>GC37</td>
<td>Gran Canaria: Degollada de Osorio, 875 m</td>
</tr>
<tr>
<td>GC39</td>
<td>Gran Canaria: Cumbre: Roque Redondo, 1900 m</td>
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</tbody>
</table>

*L. osorio* Machado, 2012

<table>
<thead>
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<th>Code</th>
<th>Location Description</th>
</tr>
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<tr>
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<td>Gran Canaria: Valsendero: Bco. Oscuro, 900 m</td>
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<tr>
<td>GC40</td>
<td>Gran Canaria: Valsendero: Bco. Cazadores, 1080 m</td>
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<tr>
<td>GC41</td>
<td>Gran Canaria: Las Huertecillas, 650 m</td>
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<td></td>
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<td>----</td>
<td>----</td>
</tr>
<tr>
<td>GC42</td>
<td>Gran Canaria: Bco. de la Mina, 1200 m</td>
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<tr>
<td><em>L. vicinus</em> Lindberg, 1953</td>
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Table 2 - Mean pairwise genetic distances between mitochondrial species groups (n=76 alleles) within *Laparocerus tessellatus* complex in the Canary Islands. Fre = *L. freyi*, Lap = *Laparocerus* sp.1, Bim = *L. bimbache*, Tess = *L. tessellatus*, Atir = *L. sp. aff. tirajana*, Tir = *L. tirajana*, Mic = *L. microphthalmus*, Oso = *L. osorio*, Obs = *L. obsitus*.

<table>
<thead>
<tr>
<th></th>
<th>Fre</th>
<th>Lap</th>
<th>Bim</th>
<th>Tess</th>
<th>ATir</th>
<th>Tir</th>
<th>Mic</th>
<th>Oso</th>
<th>Obs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncorrected p-distances (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fre</td>
<td>4.5 (2.7)</td>
<td>4.35</td>
<td>4.17</td>
<td>2.77</td>
<td>4.60</td>
<td>4.80</td>
<td>3.83</td>
<td>4.46</td>
<td>4.34</td>
</tr>
<tr>
<td>Lap</td>
<td>8.1</td>
<td>8 (3.9)</td>
<td>4.3</td>
<td>4.6</td>
<td>3.6</td>
<td>3.6</td>
<td>4.6</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Bim</td>
<td>7.6</td>
<td>8.9</td>
<td>7 (3.3)</td>
<td>4.3</td>
<td>5.2</td>
<td>5.2</td>
<td>3.8</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Tess</td>
<td>4.6</td>
<td>8.7</td>
<td>7.9</td>
<td>5.6 (3.3)</td>
<td>4.7</td>
<td>4.8</td>
<td>4.0</td>
<td>4.8</td>
<td>4.5</td>
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<tr>
<td>ATir</td>
<td>8.7</td>
<td>6.9</td>
<td>10.6</td>
<td>9.0</td>
<td>2 (1.6)</td>
<td>1.7</td>
<td>4.0</td>
<td>4.3</td>
<td>3.7</td>
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<tr>
<td>Tir</td>
<td>9.1</td>
<td>6.9</td>
<td>10.6</td>
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<td>2.3</td>
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<td>4.0</td>
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<td>0.9 (0.8)</td>
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<td>Oso</td>
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<td>9.0</td>
<td>9.9</td>
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<td>8.7</td>
<td>2.1</td>
<td>0.8 (0.7)</td>
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<tr>
<td>Obs</td>
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<td>8.8</td>
<td>7.7</td>
<td>8.6</td>
<td>6.2</td>
<td>6.9</td>
<td>2.2</td>
<td>3.6</td>
<td>2.7 (2.1)</td>
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</table>

Tamura Nei + G corrected (%)

<table>
<thead>
<tr>
<th></th>
<th>Fre</th>
<th>Lap</th>
<th>Bim</th>
<th>Tess</th>
<th>ATir</th>
<th>Tir</th>
<th>Mic</th>
<th>Oso</th>
<th>Obs</th>
</tr>
</thead>
</table>
| Average pairwise genetic distances within species are represented in diagonal. Values in diagonal inside brackets and above diagonal represent uncorrected genetic distances (p-distances). Values in diagonal outside brackets and under diagonal represent genetic corrected distances using the Tamura Nei model for nucleotide substitution.
Table 3 - Mean pairwise genetic distances between nuclear species groups (n=52 alleles) within *Laparocerus tessellatus* complex in the Canary Islands. Lap = *Laparocerus* sp.1, Fre = *L. freyi*, Bim = *L. bimbache*, Tess = *L. tessellatus*, Atir = *L.* sp. aff. *tirajana*, Oso = *L. osorio*, Tir = *L. tirajana*, Mic = *L. microphthalmus*, Obs = *L. obsitus*.

<table>
<thead>
<tr>
<th></th>
<th>Fre</th>
<th>Lap</th>
<th>Bim</th>
<th>Tess</th>
<th>ATir</th>
<th>Tir</th>
<th>Mic</th>
<th>Oso</th>
<th>Obs</th>
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<tbody>
<tr>
<td><strong>Uncorrected p-distance (%)</strong></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Fre</td>
<td>0.9 (0.9)</td>
<td>1</td>
<td>0.7</td>
<td>0.9</td>
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<td>1.8</td>
<td>2.5</td>
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<td>1.9</td>
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<tr>
<td>Lap</td>
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<td>1.8</td>
<td>2.5</td>
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<td>Tir</td>
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<td>1.9</td>
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<td>0.3 (0.3)</td>
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<tr>
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<td>0.3 (0.3)</td>
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<tr>
<td>Oso</td>
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<td>1.4</td>
<td>1.2</td>
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<td>1</td>
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<td>0.7 (0.7)</td>
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<tr>
<td>Obs</td>
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<td>1.9</td>
<td>2.1</td>
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<td>1.3</td>
<td>1.7</td>
<td>1</td>
<td>0.8 (0.8)</td>
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</table>

**TamuraNei + G corrected (%)**

Average pairwise genetic distances within species are represented in diagonal. Values in diagonal inside brackets and above diagonal represent uncorrected genetic distances (p-distances). Values in diagonal outside brackets and under diagonal represent genetic corrected distances using the Tamura Nei model for nucleotide substitution. n/a – no variation within *L. bimbache* (unique haplotype).
Table 4 - Estimated relative times in million years ago (Ma) of *Laparocerus tessellatus* complex mitochondrial lineages expressed as mean values with 95% highest posterior density (HPD) intervals. ET = earliest possible time; MRT = most recent possible time.

Island codes: EH- El Hierro, GC-Gran Canaria, LP– La Palma, TF-Tenerife. La Palma lineages= Lap-2 and Lap-3; El Hierro lineages = Bim-1 and Bim-2.

<table>
<thead>
<tr>
<th>Node</th>
<th>Description</th>
<th>Mean value (Ma)</th>
<th>95% HPD</th>
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<tr>
<td>A</td>
<td>root of the tree</td>
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<td>4.45-8.36</td>
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<td>B</td>
<td>Ingroup</td>
<td>2.71</td>
<td>2.08-3.38</td>
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<tr>
<td>C</td>
<td>Divergence of LP sequences from GC sequences</td>
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<td>0.66-1.28</td>
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<tr>
<td>D</td>
<td>ET for colonisation of LP from TF sequences (Lap-2)</td>
<td>0.21</td>
<td>0.06-0.4</td>
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<td>E</td>
<td>MRT for colonisation of LP from TF sequences (Lap-2)</td>
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<td>0.02-0.16</td>
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<td>F</td>
<td>ET for colonisation of LP from TF sequences (Lap-3)</td>
<td>0.98</td>
<td>0.63-1.35</td>
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<tr>
<td>G</td>
<td>MRT for colonisation of LP from TF sequences (Lap-3)</td>
<td>0.38</td>
<td>0.21-0.55</td>
</tr>
<tr>
<td>H</td>
<td>ET for colonisation of EH from LP sequences (Bim-1)</td>
<td>0.29</td>
<td>0.15-0.45</td>
</tr>
<tr>
<td>I</td>
<td>MRT for colonisation of EH from LP sequences (Bim-1)</td>
<td>0.12</td>
<td>0.02-0.23</td>
</tr>
<tr>
<td>J</td>
<td>ET for colonisation of EH from GC sequences (Bim-2)</td>
<td>0.4</td>
<td>0.14-0.71</td>
</tr>
<tr>
<td>K</td>
<td>MRT for colonisation of EH from GC sequences (Bim-2)</td>
<td>0.08</td>
<td>0.015-0.19</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. Distribution of sampling sites in the Canary Islands; a complete list of the site names, species collected and number of individuals can be found in Table 1. Non-consecutive numbering is for consistency with an unpublished database. Geographic coordinates (XY) for each site can be found in Table S1. Numbers in parentheses refer to the proposed maximum estimated geological ages of the islands in millions of years ago (Ma).

Figure 2. Bayesian phylogenetic tree inferred from mtDNA COII sequences (633 bp) for the Canary Islands *Laparocerus tessellatus* complex using MrBayes with the GTR+G model of sequence evolution. The tree is rooted with *L. vicinus*. Bayesian posterior probabilities are shown above nodes. Italic numbers at selected nodes indicate BEAST estimated ages of divergence with 95% highest posterior density intervals (see Materials and Methods). Letters and numbers immediately to the right of species names correspond to island codes and sampling sites, see Table 1. Shared haplotypes are highlighted in bold. A full version of the tree with all sampled individuals is provided in Fig. S1 (Supporting Information).

Figure 3. Bayesian phylogenetic tree inferred from ITS2 nuclear gene sequences (411 bp) for the Canary Islands *Laparocerus tessellatus* complex using MrBayes with the GTR+G model of sequence evolution. The tree is rooted with *L. vicinus*. Bayesian posterior probabilities are shown above nodes. Letters and numbers immediately to the right of species names correspond to site codes, see Table 1. Shared haplotypes are
highlighted in bold. A full version of the tree with all sampled individuals is provided in Fig.S2 (Supporting Information).

Figure 4. Haplotype network corresponding to the ITS2 sequence variation in the less divergent group of the *Laparocerus tessellatus* complex (clade C in Fig 3, see text for more details) in the Canary Islands. Colours correspond to islands, and differently coloured segments within circles represent haplotype sharing across islands. The Gran Canarian outgroup *L. osorio* is represented by green haplotypes 48, 49 and 50. Lines represent a mutational step; black circles represent missing or unsampled haplotypes.

Figure 5. Interisland colonisation history within the *Laparocerus tessellatus* complex inferred from DNA sequence data from the mitochondrial COII gene and the nuclear ITS2 gene. The islands of La Palma and El Hierro are inferred to have been colonised from more than one island, suggesting a complex history involving genetic admixture between different founding species for the origin of the single species occurring on each island (see text for details).
Figure 1
Figure 2

Key:
- La Palma
- Gran Canaria
- Tenerife
- El Hierro

Evolutions/ancestors:
- H. L. tenalis
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Figure 3
Figure 5