

**MATERNAL GENETIC STRUCTURE
REVEALS AN INCIPIENT DIFFERENTIATION
IN THE CANARY ISLANDS CHIFFCHAFF
*PHYLLOSCOPUS CANARIENSIS***

**DIFERENCIACIÓN GENÉTICA INCIPIENTE
EN EL MOSQUITERO CANARIO *PHYLLOSCOPUS CANARIENSIS***

Juan Carlos ILLERA¹ *, Álvaro RAMÍREZ², Laura RODRÍGUEZ¹,
Konstantinos POLYPATHELLIS¹ and Javier PÉREZ-TRIS²

SUMMARY.—The Canary Islands are characterised by an outstanding level of biodiversity with a high number of endemic taxa. The Canarian avifauna is no exception and six extant avian species are recognised as endemic. However, we have a limited understanding of the genetic structure of these taxa, which makes it difficult to identify conservation priorities based on the existence of unique lineages. We analysed the diversification process and demography of the Canary Islands Chiffchaff *Phylloscopus canariensis* using two mitochondrial genes (cytochrome b and cytochrome oxidase subunit I). Although the species colonised the Canary Islands during the early Pleistocene, our results suggest a recent process of differentiation, which ran in parallel to the Last Glacial Maximum. Chiffchaffs from Gran Canaria emerge as a unique lineage since none of the haplotypes found on this island were recorded anywhere else in the archipelago. Our findings suggest a process of divergence according to the gradual colonisation and subsequent isolation of nearby islands. Demographic results show a stable trend of Chiffchaff populations until the colonisation of Gran Canaria (ca. 15,000 years ago), where the species significantly increased its effective population size. Nowadays, the effective population size of the Canary Islands Chiffchaff is stable, which highlights the ability of Chiffchaffs to adapt to local disturbances related to human activities. Overall, our results provide a scenario of incipient differentiation of the Canary Islands Chiffchaff and, importantly, underscore once more the role of Gran Canaria in driving speciation processes in this archipelago.—Illera, J.C., Ramírez, A., Rodríguez, L., Polypathellis, K. & Pérez-Tris, J. (2020). Maternal genetic structure reveals an incipient differentiation in the Canary Islands Chiffchaff *Phylloscopus canariensis*. *Ardeola*, 67: 401-414.

Key words: avian evolution, demography, Gran Canaria, island biogeography, island phylogeography, Macaronesia, oceanic islands, population genetics.

RESUMEN.—Las Islas Canarias se caracterizan por albergar una excepcional biodiversidad con multitud de taxones endémicos, y las aves no son una excepción. En la actualidad se reconocen hasta seis

¹ Research Unit of Biodiversity (UO-CSIC-PA), Oviedo University, Mieres, Asturias, Spain.

² Department of Biodiversity, Ecology and Evolution, Complutense University of Madrid, Spain.

* Corresponding author: illerajuan@uniovi.es / jcillera3@hotmail.com

especies de aves endémicas. Sin embargo, la información disponible sobre la estructura genética que mantienen estas especies dentro del archipiélago es muy limitada. Con el presente trabajo estudiamos el proceso de diferenciación y la evolución demográfica del mosquitero canario *Phylloscopus canariensis*, usando dos genes mitocondriales (citocromo b y citocromo oxidasa subunidad I). Los resultados muestran que, a pesar que la especie habría colonizado el archipiélago durante el Pleistoceno temprano, el proceso de diferenciación no habría comenzado hasta los últimos 30.000 años. Solo la isla de Gran Canaria, la cual habría sido colonizada durante el Pleistoceno tardío (ca. 15.000 años), mantiene haplotipos no compartidos con otra isla. El análisis de aislamiento por distancia sugiere un patrón de colonización y aislamiento posterior acorde a un modelo gradual, en donde las islas más cercanas son también genéticamente más similares. El análisis demográfico muestra una tendencia estable en el tamaño efectivo poblacional, hasta el momento en que la isla de Gran Canaria es colonizada. En ese momento el tamaño efectivo se incrementó significativamente hasta el Holoceno medio (ca. 5.000 años), donde alcanzó de nuevo un tamaño estable. Este resultado sugiere una gran plasticidad ecológica de esta especie, que ha sido capaz de superar múltiples perturbaciones como ha sido la pérdida de grandes masas forestales desde la llegada del ser humano a Canarias. En resumen, nuestros resultados muestran un proceso incipiente de diferenciación del mosquitero canario en el archipiélago, y resalta de nuevo el papel significativo de Gran Canaria a la hora de promover linajes únicos. — Illera, J.C., Ramírez, A., Rodríguez, L., Polypathellis, K. y Pérez-Tris, J. (2020). Diferenciación genética incipiente en el mosquitero canario *Phylloscopus canariensis*. *Ardeola*, 67: 401-414.

Palabras clave: demografía, especiación aviar, filogeografía, genética de poblaciones, Gran Canaria, islas oceánicas, Macaronesia.

INTRODUCTION

The avifauna of the Canary Islands has fascinated naturalists and biologists since the nineteenth century due to the diversity of breeding species and, especially, to their conspicuous phenotypic differentiation in relation to their mainland counterparts. This interest resulted in the description of a plethora of new endemic species and subspecies during the nineteenth and twentieth centuries (Martín & Lorenzo, 2001). Taxonomic descriptions were traditionally performed by visual inspection of individuals, with subsequent identification of diagnostic traits. However, this approach was not associated with rigorous statistical analyses, which raises doubts about whether these populations are idiosyncratically distinct (Illera *et al.*, 2016). In recent years, the routine use of molecular tools has provided insights into our understanding of the genetic diversity, colonisation pathways and taxonomic identity of many taxa in the Canary Islands (Juan *et al.*,

2000; Stervander *et al.*, 2015; Kangas *et al.*, 2018; Senfeld *et al.*, 2020). Some of these findings have revealed strikingly high genetic differentiation between nearby populations, which is consistent with subspecific classification (Dietzen *et al.*, 2003, 2015; Päckert *et al.*, 2006). These isolated and genetically distinct populations probably went unnoticed because they showed similar morphological traits (Kvist *et al.*, 2005; Illera *et al.*, 2018). Overall, these findings highlight the need for a rigorous assessment of avian biodiversity in the Canary Islands to identify overlooked cryptic diversity, which should be officially recognised under Spanish and Canarian conservation laws. The final aim should be to support the management of all biological resources according to their conservation status.

Over the last two decades, knowledge of the evolutionary history of many avian taxa in the Canary Islands has noticeably increased thanks to a combination of phylogenetic or phylogenomic data and fossil information

(reviewed in Illera *et al.*, 2012, 2016; Van Doren *et al.*, 2017). However, we have a limited understanding at the population level of the genetic structure of most endemic bird species that occur in the Canary Islands. This knowledge gap precludes recognising new cryptic lineages, which should be a critical factor in conservation management (Illera *et al.*, 2007, 2016).

The Canary Islands Chiffchaff *Phylloscopus canariensis* is one of six endemic avian species that inhabits the Canary Islands. It is a small (<10g) sedentary passerine (González-Melián, 2018) that occurs on the central and western islands, and is one of the most common land species that breeds in the Canary Islands (Martín & Lorenzo, 2001; González-Melián, 2007). Although the species is absent from the eastern islands (i.e., Fuerteventura and Lanzarote), a recognised (extinct) endemic subspecies (*P. c. exsul*) was breeding on these two islands

probably until the first quarter of the twentieth century (Figure 1). Chiffchaffs show wide ecological plasticity in with respect to climatic conditions. Thus, the species inhabits vegetated areas ranging from sea level to alpine habitats (>2,400m a.s.l.), being absent only from sparsely vegetated areas (Martín & Lorenzo, 2001).

Recent phylogenetic analyses have indicated that the Canary Islands Chiffchaff is related to the Mountain Chiffchaff *P. sibilans*, a species with a Caucasian distribution (Tietze *et al.*, 2015, but see Alström *et al.*, 2018). This result is remarkable because the distributions of other chiffchaff species are geographically closer to the Canary Islands than that of the Mountain Chiffchaff. Thus, the Common Chiffchaff (*P. collybita*) and the Iberian Chiffchaff (*P. ibericus*) are common breeding and/or wintering species in northern Africa and the Iberian Peninsula (Gordo *et al.*, 2017). However, according to

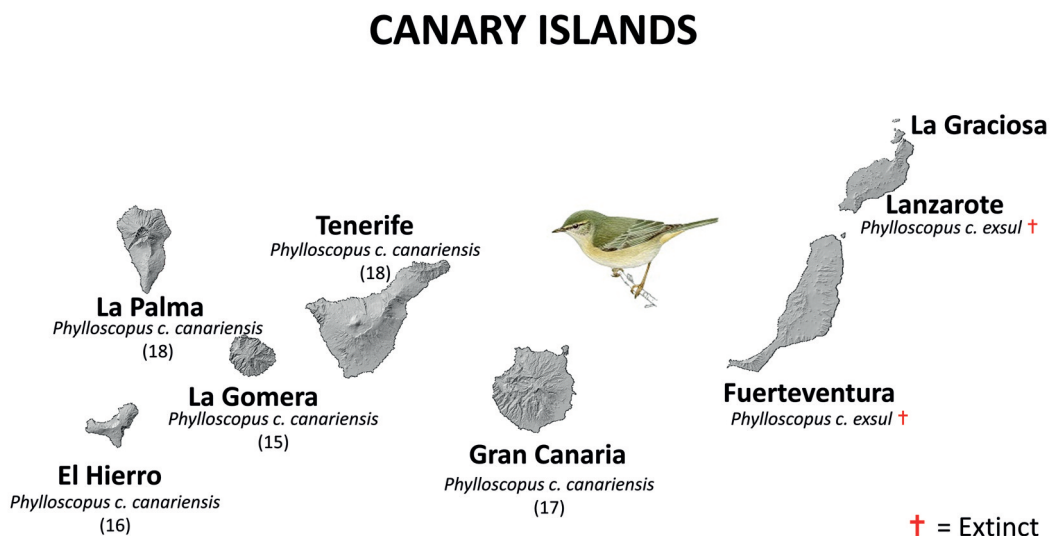


FIG. 1.—Distribution of the extant and extinct populations of the Canary Islands Chiffchaff *Phylloscopus canariensis*. Sample size per island given in parentheses.

[Mapa de Canarias mostrando la distribución de las poblaciones actuales y extintas del mosquitero canario *Phylloscopus canariensis*. El tamaño de muestra analizado por cada isla se muestra entre paréntesis.]

the limited nodal support found in both phylogenies, the phylogenetic relationships within this clade do not yet appear to have been resolved (Tietze *et al.*, 2015; Alström *et al.*, 2018). In fact, until the beginning of the twenty-first century, the Canary Islands Chiffchaff was considered to be a subspecies of the Common Chiffchaff (Martín & Lorenzo, 2001). However, significant differentiation found in mitochondrial sequences, and acoustic and morphological data between taxa of the Chiffchaff complex (i.e., *P. ibericus*, *P. canariensis* and *P. sindianus*) supported a new taxonomical classification in which the Canary Islands Chiffchaff emerged as a species in its own right (Helbig *et al.*, 1996; Raković *et al.*, 2019).

The chiffchaffs colonised the Canary Islands during the early Pleistocene, 2.28 million years ago (Valente *et al.*, 2017). According to its taxonomic classification, the Canary Islands Chiffchaff is a polytypic species with two subspecies (*P. c. canariensis* and *P. c. exsul*), which means that individuals of each subspecies experienced reproductive isolation from one another. However, it is also plausible to expect further differentiation, especially between the central and western islands, as has been found in other avian species. Thus, distinctive lineages have emerged for a wide number of taxa on Gran Canaria, including European Robins *Erithacus rubecula*, Common Chaffinches *Fringilla coelebs*, Blue Chaffinches *F. polatzeki* and Canarian Blue Tits *Cyanistes teneriffae* (Pestano *et al.*, 2000; Dietzen *et al.*, 2003; Kvist *et al.*, 2005; Suárez *et al.*, 2009).

Here, we provide a comprehensive phylogeographic framework based on two mitochondrial genes: cytochrome b (cyt-b) and cytochrome oxidase subunit I (COI). Our approach had three aims. The first goal was to scrutinise the genetic structure of the Canary Islands Chiffchaff across the central and western islands, with the specific aim of estimating the genetic variation and relation-

ships among islands. Based on the taxonomic hierarchy recognised to date (there is only one extant subspecies), we predicted that there is contemporary gene flow among current populations and an absence of significant genetic structure. The second goal was to determine the pathway of colonisation and phylogenetic relationships of the Canarian populations. Here we expected to find a very recent differentiation process, in which genetic differentiation increases with geographical distances among populations. Finally, our third objective was to analyse the demographic evolution of the Canary Islands Chiffchaffs, with the final aim of determining the role of human arrival on its effective population size. Because the Canary Islands Chiffchaff is a common avian species inhabiting woodland habitats, we predicted that the reduction of forest habitats, that began immediately after humans arrived in the archipelago (especially after the European conquest), should have resulted in a significant reduction of the effective population size.

MATERIAL AND METHODS

Sampling

Individuals (n = 86) were caught at various localities, using mist nets, between 2003 and 2010. We ringed all individuals with unique numbered aluminium rings. We collected blood samples ($\approx 25\mu\text{l}$) by brachial or jugular venipuncture using insulin syringes (0.5mm), and diluted them in 1000 μl of pure ethanol in screw-cap microfuge tubes. Finally, we released all birds at the site of capture.

Molecular procedures

We extracted DNA using an ammonium acetate method ([dx.doi.org/10.17504/PROTO](https://doi.org/10.17504/PROTO))

cols.io.knycvfw), for working with a final concentration of 10-50ng/μl. We amplified a 1018 base pair (bp) fragment of the mitochondrial *cyt-b* gene using primers L14995 (5' CTCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG 3') and H16065 (5' CTAAGAAGGGTGGAGTCTTCAGTT TTTGGTTTACAAGAC 3') (Helbig *et al.*, 1995). PCR reactions were set up in a final volume of 10μL including 5μL of GoTaq® Green Master Mix 2× (Promega Corporation, Madison, WI, USA), 0.5μL (10mM) of primers L14995 and H16065 and 1μL of DNA. Reactions were performed on a G-Storm GS2 thermal cycler (Somerton Biotechnology Centre, Somerset, UK) under the following conditions: initial denaturation at 94°C for 4 minutes followed by 35 denaturation cycles at 94°C for 30s, annealing at 50°C for 45s, and extension at 72°C for 60s, and a final extension at 72°C for 10min. PCR products were checked by electrophoresis in 1.5% agarose gels stained with GelRed™ nucleic acid gel stain (Biotium, Inc., Hayward, CA, USA). The samples were purified using the E.Z.N.A. Gel Extraction Kit OMEGA. Sequencing reactions were performed from both ends using the Perkin Elmer BigDye v. 3.1 (Applied Biosystems, Carlsbad, CA, USA) terminator reaction mix in a volume of 10μL using 1μL of PCR product and L14995 and H16065 primers. Sequencing reaction conditions were: initial denaturation at 94°C for 2 minutes followed by 25 denaturation cycles at 94°C for 30s, annealing at 50°C for 30s, and extension at 60°C for 2 minutes, followed by a final extension at 60°C for 1 minute. The final product was sequenced on an ABI PRISM® 3130xl Genetic Analyzer.

We also amplified a 615 bp fragment of the mitochondrial COI gene using the primers PasserF1 (5' CCAACCACAAAGACATCGGAACC 3') and PasserR1 (5' GTA AACTTCTGGGTGACCAAAGAATC 3') (Lohman *et al.*, 2009). PCR reactions were performed using the following conditions:

initial denaturation at 94°C for 3 minutes followed by 35 denaturation cycles at 94°C for 1 minute, annealing at 58°C for 1 minute, extension at 72°C for 1.5 minutes, and a final extension at 72°C for 10 minutes. Sequencing reactions were performed with the same reagents and conditions that were used with the *cyt-b* gene.

Statistical analyses

We aligned sequences by hand using BioEdit version 7.0.9 (Hall, 1999) and Chromas version 2.5.1. (<https://technelysium.com.au/wp/chromas/>) programmes. We used the DnaSP programme (Version 5.10.01; Librado & Rozas, 2009) to calculate the genetic diversity estimated per population (island), in terms of the number of haplotypes, haplotype and nucleotide diversities and theta ($2Ne\mu$). We assessed genetic distances between populations using pairwise F_{ST} values and tested their statistical significance by performing 10,000 permutations using the ARLEQUIN programme (version 3.5.1.2; Excoffier & Lischer, 2010). We also evaluated isolation by distance by testing whether pairwise genetic distances were correlated with geographic distances between islands, for which we performed a Mantel test using the vegan package (Oksanen *et al.*, 2018) in the R programme (Version 3.5.1; R Core Team, 2018). We used Rousset's approach, which compares the F_{ST} pairwise genetic distance matrix ($F_{ST} / (1 - F_{ST})$) with log-transformed geographic distance (Rousset, 1997).

We inferred the network of relationships among mitochondrial sequences (both *cyt-b* and COI) using the TCS programme (Version 1.21; Clement *et al.*, 2000), which implements the probabilistic method of statistical parsimony. We used a 5% limit, and ambiguity characters were treated as missing data. The graph file obtained in TCS was then used to classify haplotypes and display

the final network using the TCS Beautifier programme (tcsBU) (Santos *et al.*, 2016).

We estimated population divergence times using the BEAST programme (Version 1.8.1; Drummond *et al.*, 2012). We only included the sequences of *cyt-b*, which is the most commonly used mitochondrial gene for dating divergence times in avian taxa (Valente *et al.*, 2017, 2020). We inferred the

most appropriate nucleotide substitution model (HKY + I) according to the JModelTest programme (Version 2.1.4; Darrriba *et al.*, 2012). We used a strict molecular clock, with a mean of 0.01 and standard deviation of 0.0075 substitutions per site per million years (Illera *et al.*, 2008). We ran two independent MCMC analyses of 50,000,000 generations, with a burn-in of 5,000,000 steps. Finally,

TABLE 1

Genetic diversity estimates from cytochrome b and COI genes for the Canary Islands Chiffchaff *Phylloscopus canariensis*. N: Sample size. Hap: Number of haplotypes. HD: Haplotype diversity and standard deviation (SD). ND: Nucleotide diversity and standard deviation (SD). θ : Theta ($2Ne\mu$), where Ne is the effective population size and μ the mutation rate.

[*Estimas de diversidad genética del mosquitero canario Phylloscopus canariensis obtenidas con los genes mitocondriales citocromo b y citocromo oxidasa subunidad I. N: Tamaño de muestra. Hap: Número de haplotipos. HD: Diversidad haplotípica y desviación estándar (SD). ND: Diversidad nucleotídica y desviación estándar (SD). θ : Theta ($2Ne\mu$), donde Ne es el tamaño efectivo poblacional y μ es la tasa de mutación.*]

Populations	Cytochrome b (Cyt-b)						
	N	Hap	HD	SD	ND	SD	θ
Total	84	33	0.943	0.012	0.00683	0.00056	0.00056
Gran Canaria	17	7	0.794	0.078	0.00188	0.00038	0.00291
Tenerife	18	11	0.889	0.064	0.00507	0.00142	0.00771
La Gomera	15	9	0.914	0.052	0.00236	0.00042	0.00363
La Palma	18	9	0.797	0.090	0.00251	0.00052	0.00343
El Hierro	16	5	0.767	0.080	0.00224	0.00044	0.00237
Populations	Cytochrome oxidase subunit I (COI)						
	N	Hap	HD	SD	ND	SD	θ
Total	86	11	0.889	0.064	0.00507	0.00142	0.00771
Gran Canaria	17	3	0.691	0.052	0.00146	0.00018	0.00096
Tenerife	18	11	0.954	0.026	0.00547	0.00072	0.00709
La Gomera	15	5	0.810	0.059	0.00208	0.00031	0.00200
La Palma	18	5	0.771	0.063	0.00198	0.00033	0.00189
El Hierro	18	4	0.542	0.123	0.00132	0.00046	0.00189

we evaluated the convergence of the chains with Tracer (Version 1.7.1; Rambaut *et al.*, 2018). Recently, two studies have revealed problems with the joint estimation of tree topology provided by BEAST (Emerson & Hickerson, 2015; Emerson *et al.*, 2015). For this reason, we performed another Bayesian phylogenetic inference using the Mr. Bayes programme (Version 3.1.2; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). We ran Mr. Bayes for 10,000,000 iterations, and we sampled trees and model parameters every 100 generations. We set up a burn-in of 2,500,000 generations, and performed two independent runs to ensure stability in the posterior probabilities. We also assessed the convergence of the MCMCs obtained with Mr. Bayes using Tracer.

We evaluated the changes in the effective population size (N_e) of the Canary Islands Chiffchaffs using the Bayesian skyline plot implemented in BEAST (Drummond *et al.*, 2012). This plot is a coalescent demographic reconstruction, which is effective for detecting population expansions, contractions and bottleneck events.

RESULTS

In total, we obtained 170 sequences from *cyt-b* ($n = 84$) and COI ($n = 86$) genes (15-18 sequences per population, Table 1). Unique haplotypes have been deposited in the NCBI gene bank database (MT237934-52; MT241268-241300). Cytochrome b showed higher genetic diversity, with three times as many haplotypes ($n = 33$) as COI ($n = 11$). Tenerife and La Gomera exhibited the highest values of genetic diversity both for haplotype and nucleotide diversity, while the most peripheral populations (El Hierro and Gran Canaria) provided the lowest estimates (Table 1).

Haplotype networks for *cyt-b* and COI were concordant (Figure 2 and Supplementary Material, Appendix 1, Figure A1), depicting a limited differentiation in the Canary Islands Chiffchaffs. Gran Canaria was the only island for which no haplotypes were found to be shared with other populations. The parsimony network based on *cyt-b* showed the La Gomera population connecting the two most frequent haplotypes, which were found in individuals from three and four islands.

TABLE 2

Cytochrome b pairwise F_{ST} values obtained in the Canary Islands Chiffchaff *Phylloscopus canariensis*. P values obtained after 10,000 permutations are indicated with asterisks. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

[Valores F_{ST} de las comparaciones por pares entre las diferentes poblaciones de mosquitero canario *Phylloscopus canariensis*. El valor de significación P se obtiene tras 10.000 permutaciones y se indica con asterisco. * = $P < 0,05$; ** = $P < 0,01$; *** = $P < 0,001$.]

	El Hierro	Gran Canaria	La Gomera	La Palma
El Hierro				
Gran Canaria	0.843***			
La Gomera	0.076*	0.846***		
La Palma	0.233**	0.849***	0.213**	
Tenerife	0.112**	0.718***	0.143**	0.304***

All pairwise F_{ST} values were significant ($P < 0.05$), and all pairwise F_{ST} values including Gran Canaria reached the highest differentiation levels with high statistical support ($P < 0.001$) (Table 2). Finally, pairwise F_{ST} and geographical distances were positively and significantly correlated (Mantel statistic $r = 0.761$, $P = 0.033$) (Figure 3).

Bayesian analyses confirmed the incipient isolation of the Gran Canaria population, with all individuals grouping together. However, two individuals from Tenerife also grouped with the Gran Canarian birds within the same pool with high nodal support (Posterior probability = 0.97). The remaining individuals pooled in different groups but

without showing a clear differentiation among islands (Supplementary material appendix 1, Figure A2).

The differentiation process of the extant Canary Islands Chiffchaff populations started recently, at the end of Pleistocene (29,900 years; 22,900-37,000 years ago, lower and upper 95% highest posterior density values, respectively). Gran Canaria began its genetic divergence from the remaining populations shortly after the extant chiffchaffs started to differentiate (14,900 years; 9,681-20,040 years ago).

Our Bayesian Skyline reconstruction showed a population size history in which the Canary Islands Chiffchaffs experienced

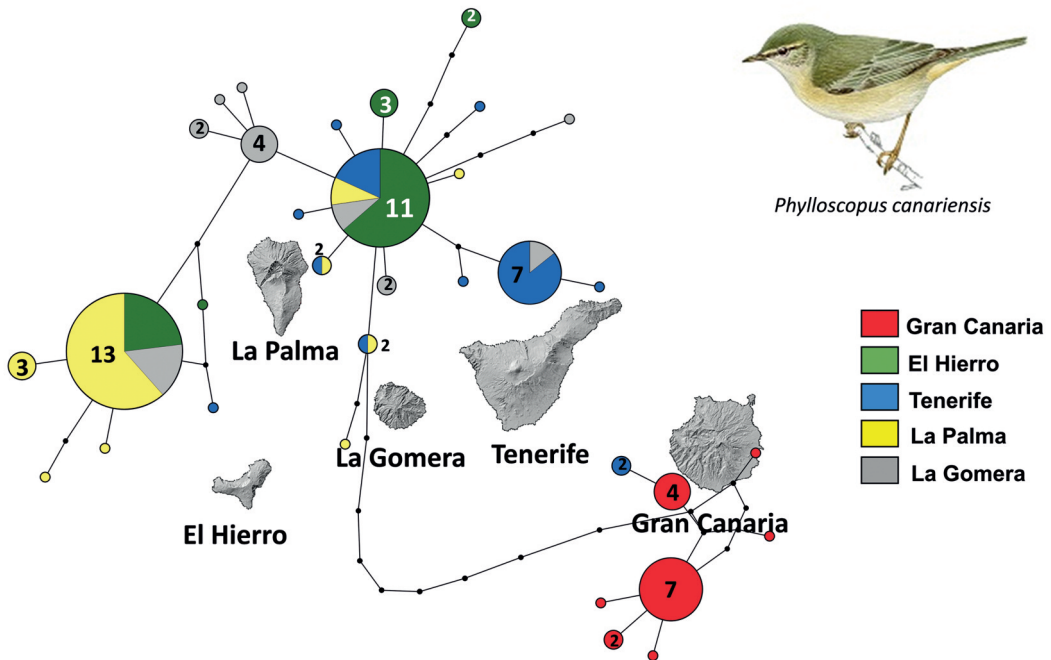


FIG. 2.—Parsimony network of the Canary Islands Chiffchaff *Phylloscopus collybita* derived using the TCS programme, based on cytochrome b sequences. The circle sizes represent the number of individuals that shared each haplotype, while numbers indicate how many individuals were sequenced with that haplotype.

[Red de haplotipos obtenido con el programa TCS usando secuencias del citocromo b. El tamaño de los círculos representa el número de individuos compartiendo cada haplotipo. Los números indican el número de individuos secuenciados con ese haplotipo.]

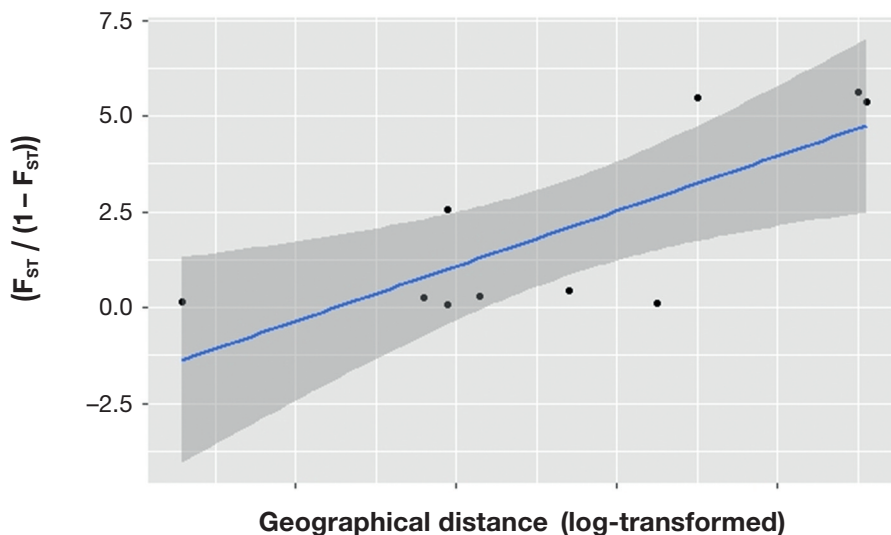


FIG. 3.—Isolation by distance relationship for the Canary Islands Chiffchaff *Phylloscopus canariensis*. Pairwise genetic values ($F_{ST} / (1 - F_{ST})$) are plotted against geographical distances (log-transformed). [Análisis de aislamiento por distancia para el mosquitero canario *Phylloscopus canariensis*. Las distancias genéticas ($F_{ST} / (1 - F_{ST})$) están representadas en relación con las distancias geográficas transformadas logarítmicamente.]

a demographic expansion event coincident with the divergence of Gran Canaria (*ca.* 15,000 years ago). This expansion finished approximately 5,000 years ago, and nowadays the species shows a stable trend (Figure 4). Assuming a generation time of two years for the Canary Islands Chiffchaff, we estimated an effective population size (N_e) of 200,736 individuals (61,130–525,048 birds, lower and upper 95% highest posterior density values, respectively).

DISCUSSION

Although chiffchaffs colonised the Canary Islands during the early Pleistocene (Valente *et al.*, 2017), our results showed an incipient differentiation process that ran in parallel with the Last Glacial Maximum. The ongoing process of differentiation was inferred from three independent analyses:

1) haplotype networks; 2) significant pairwise F_{ST} values found among all populations; and 3) the Bayesian inference obtained with BEAST and Mr. Bayes. Overall, our findings suggest a recent differentiation mirroring a process of incomplete lineage sorting with a limited contemporary gene flow among extant populations. The fact that two individuals from Tenerife appeared nested with the Gran Canaria cluster, and the limited gene flow supported by F_{ST} values, could represent the retention of an ancient polymorphism from the ancestor of the Canary Islands Chiffchaff. It is interesting that acoustic information published to date also suggests a similar pattern of differentiation. For example, Henning *et al.* (1994) found conspicuous differences in some acoustic traits; specifically, singing and alarm calls (*i.e.*, contact calls) were found to differ among some populations. They distinguished two main groups: one group pooled individuals from Gran Canaria and

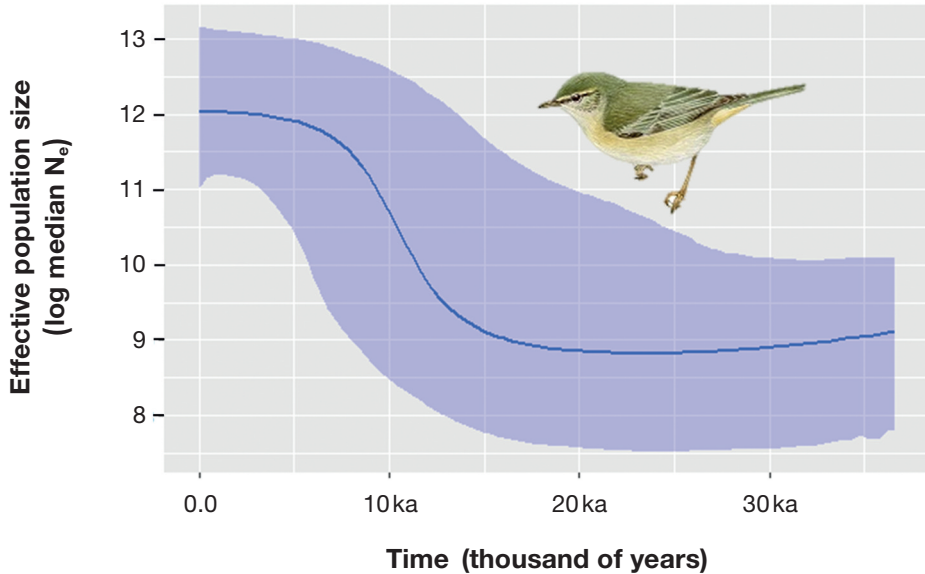


FIG. 4.—Demographic reconstruction of the Canary Islands Chiffchaff *Phylloscopus canariensis* obtained from all extant populations, produced from a Bayesian skyline analysis performed with BEAST 1.8. Median population size and 95% HPD intervals are plotted over time. The y-axis represents effective population size N_e (log transformed).

[Reconstrucción demográfica del mosquitero canario *Phylloscopus canariensis* en las islas Canarias usando un análisis bayesiano implementado en el programa BEAST 1.8. La mediana del tamaño efectivo poblacional, así como los intervalos más altos y más bajos del 95% de la densidad posterior más alta (HPD) son representados a lo largo del tiempo. El eje y representa el tamaño efectivo poblacional absoluto transformado logarítmicamente.]

Tenerife, and the other group pooled individuals from the remaining populations. In addition, to a lesser extent, these authors also found differences between all Canarian islands. In addition, Naguib *et al.* (2001) studying chiffchaff calls in Tenerife and La Gomera found significant differences between these islands. Taken together, these acoustic findings suggest a context of limited dispersal events between nearby islands.

There was no evident relationship between the Last Glacial Maximum and divergence events, but a similar pattern of an early Pleistocene colonisation event and recent differentiation has also been found in Berthelot's Pipit *Anthus berthelotii* in Macaronesia (Illera *et al.*, 2007; Spurgin *et*

al., 2014). We have a limited understanding about how Quaternary climatic oscillations have created or extirpated genetic diversity through the contraction, expansion and extinction of Canarian biota in the archipelago (García-Verdugo *et al.*, 2019). However, it is plausible to speculate that the climate changed towards drier conditions during the late Pleistocene, although with episodes of wetter periods (Alonso-Zarza & Silva, 2002; Weigelt *et al.*, 2016), which favoured the expansion of some avian taxa in Macaronesia.

The divergence process found in Gran Canaria, where the population appears to be isolated from the remaining Canary Islands Chiffchaff populations, is remarkable. This genetic finding highlights once more the role

of Gran Canaria in providing distinctive lineages of Canarian avifauna. The level of genetic differentiation is lower than that observed in other Canarian species, such as Robins, Canarian Blue Tits, and Common and Blue Chaffinches (Pestano *et al.*, 2000; Dietzen *et al.*, 2003; Stervander *et al.*, 2015; Illera *et al.*, 2018). However, it is similar to the level of differentiation found in the Southern Grey Shrike *Lanius meridionalis* (Padilla *et al.*, 2015), and slightly higher than that documented in the Stone-Curlew *Burhinus oedicephalus* (Mori *et al.*, 2017) and Spectacled Warbler *Sylvia conspicillata* (Illera *et al.*, 2014). Why does Gran Canaria frequently emerge as a unique lineage within Canarian birds? Gran Canaria is the third oldest island in the archipelago (14 my), the third largest island (1,560km²), and supports a wide variety of habitats, which could further favour diversification processes. Therefore, a combination of age, habitat diversity, and isolation from other islands with similar ecosystems (such as Tenerife, which is approximately 60km away), could explain the recurrent differentiation patterns observed.

The incipient differentiation process recorded with the mitochondrial sequences limits our ability to unravel the pathway of chiffchaff colonisation within the archipelago. Our findings show a positive correlation between geographical and genetic distances, suggesting gradual genetic differentiation with increasing distances. However, the incomplete lineage sorting recorded, and the absence of sequences from the extinct subspecies from the eastern islands, precludes identifying the ancestral population. A future study using high genomic coverage and wide population sampling, including the extinct taxa of the eastern islands, is now needed to infer which population acted as a source and served as the cradle of diversification for the Canary Islands Chiffchaff.

Finally, our demographic analyses suggest that the effective population size of

the Canary Islands Chiffchaff has evolved according to its diversification history in the Canary Islands. We did not record any bottleneck event in the Canary Islands Chiffchaff, which could have been attributable to the arrival of aboriginal people (*ca.* 2,000 years ago) or Europeans (*ca.* 500 years ago) (Alcover *et al.*, 2009). Instead, we found a stable trend in the effective population size from the onset of its diversification in the central islands (Tenerife and La Gomera) and western islands (La Palma and El Hierro) islands until the colonisation of Gran Canaria (*ca.* 15,000 years ago), where the effective population size of the Canary Islands Chiffchaff increased remarkably (Figure 4). This last result seems to be a logical consequence of occupying a large island such as Gran Canaria. A new equilibrium was reached 5,000 years ago, with no evidence of a drastic reduction of the effective population size thereafter. Overall, this result suggests a limited role of human arrival (either aboriginal or European) in determining the population size of the Canary Islands Chiffchaff, which could be explained by the considerable ability of chiffchaffs to adapt to local disturbances.

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AUTHOR CONTRIBUTION.—JCI designed and supervised the study. JPT, AR and JCI performed the fieldwork. JCI, LR and KP carried out the molecular work. JCI performed statistical analyses. JCI drafted the manuscript and all authors commented and approved the final version of the manuscript.

REFERENCES

- Alcover, J.A., Rando, J.C., García-Talavera, F., Hutterer, R., Michaux, J., Trias, M. & Navarro, J.F. (2009). A reappraisal of the stratigraphy of Cueva del Llano (Fuerteventura) and the chronology of the House Mouse (*Mus musculus*) introduction into the Canary Islands. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 277: 184-190.
- Alström, P., Rheindt, F.E., Zhang, R., Zhao, M., Wang, J., Zhu, X., Gwee, C.Y., Hao, Y., Ohlson, J., Jia, C., Prawiradilaga, D.M., Ericson, P.G.P., Lei, F. & Olsson, U. (2018). Complete species-level phylogeny of the leaf warbler (Aves: Phylloscopidae) radiation. *Molecular Phylogenetics and Evolution*, 126: 141-152.
- Alonso-Zarza, A.M. & Silva, P.G. (2002). Quaternary laminar calcretes with bee nests: evidences of small-scale climatic fluctuations, Eastern Canary Islands, Spain. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 178: 119-135.
- Clement, M., Posada, D. & Crandall, K. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9: 1657-1660.
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9: 772.
- Dietzen, C., Michels, J.P. & Wink, M. (2015). Formal description of a new subspecies of the European Robin from Gran Canaria Island, Spain (Aves: Muscicapidae: *Erithacus rubecula marionae* subsp. nov.). *The Open Ornithology Journal*, 8: 39-42.
- Dietzen, C., Witt, H.-H. & Wink, M. (2003). The phylogeographic differentiation of the European robin *Erithacus rubecula* on the Canary Islands revealed by mitochondrial DNA sequence data and morphometrics: evidence for a new robin taxon on Gran Canaria? *Avian Science*, 3: 115-132.
- Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29: 1969-1973.
- Emerson, B.C., Alvarado-Serrano, D.F. & Hickerson, M.J. (2015). Model misspecification confounds the estimation of rates and exaggerates their time dependency. *Molecular Ecology*, 24: 6013-6020.
- Emerson, B.C. & Hickerson, M.J. (2015). Lack of support for the time-dependent molecular evolution hypothesis. *Molecular Ecology*, 24: 702-709.
- Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567.
- García-Verdugo, C., Caujapé-Castells, J., Illera, J.C., Mairal, M., Patiño, J., Reyes-Betancort, A. & Scholz, S. (2019). Pleistocene extinctions as drivers of biogeographical patterns on the easternmost Canary Islands. *Journal of Biogeography*, 46: 845-859.
- González-Melián, E. (2007). Mosquitero Canario *Phylloscopus canariensis* (Canary Chiffchaff *Phylloscopus canariensis*). In, J.A. Lorenzo (ed.): *Atlas de las aves nidificantes en el archipiélago canario (1997-2003)*, pp. 376-379. Dirección General de Conservación de la Naturaleza-Sociedad Española de Ornitología. Madrid.
- González-Melián, E. (2018). Sex determination of the Canary Island Chiffchaff *Phylloscopus canariensis* using morphological traits and molecular sexing. *Ornithological Science*, 17: 17-34.
- Gordo, O., Arroyo, J.L., Rodríguez, R. & Martínez, A. (2017). Inability of biometry to discriminate Iberian and Common Chiffchaffs during the autumn migration period. *Ardeola*, 64: 49-65.
- Helbig, A.J., Martens, J., Seibold, I., Henning, F., Schottler, B. & Wink, M. (1996). Phylogeny and species limits in the Palearctic Chiffchaff *Phylloscopus collybita* complex: mitochondrial genetic differentiation and bioacoustic evidence. *Ibis* 138: 650-666.

- Helbig, A.J., Seibold, I., Martens, J. & Wink, M. (1995). Genetic differentiation and phylogenetic relationships of Bonelli's warbler *Phylloscopus bonelli* and green warbler *P. nitidus*. *Journal of Avian Biology*, 26: 139-153.
- Henning, F., Schottler, B. & Martens, J. (1994). Inselspezifische Rufe Kanarischer Zilpzalpe (*Phylloscopus collybita canariensis*). *Verandlungen der deutschen zoologischen Gesellschaft*, 87: 43.
- Illera, J.C., Emerson, B.C. & Richardson, D.S. (2007). Population history of Berthelot's pipit: colonization, gene flow and morphological divergence in Macaronesia. *Molecular Ecology*, 16: 4599-4612.
- Illera, J.C., Palmero, A.M., Laiolo, P., Rodríguez, F., Moreno, A.C. & Navascués, M. (2014). Genetic, morphological, and acoustic evidence reveals lack of diversification in the colonisation process in an island bird. *Evolution*, 68: 2259-2274.
- Illera, J.C., Rando, J.C., Richardson, D.S. & Emerson, B.C. (2012). Age, origin and extinctions of the avifauna of Macaronesia: a synthesis of phylogenetic and fossil information. *Quaternary Science Reviews*, 50: 14-22.
- Illera, J.C., Rando, J.C., Rodríguez-Expósito, E., Hernández, M., Claramunt, S. & Martín, A. (2018). Acoustic, genetic, and morphological analyses of the Canarian common chaffinch complex *Fringilla coelebs* ssp. reveals cryptic diversification. *Journal of Avian Biology*, 49: e01885.
- Illera, J.C., Richardson, D.S., Helm, B., Atienza, J.C. & Emerson, B.C. (2008). Phylogenetic relationships, biogeography and speciation in the avian genus *Saxicola*. *Molecular Phylogenetics and Evolution*, 48: 1145-1154.
- Illera, J.C., Spurgin, L.G., Rodríguez-Expósito, E., Nogales, M. & Rando, J.C. (2016). What are we learning on speciation and extinction from the Canary Islands? *Ardeola*, 63: 15-33.
- Juan, C., Emerson, B.C., Oromí, P. & Hewitt, G. (2000). Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology and Evolution*, 15: 104-109.
- Kangas, V., Carrillo, J., Debray, P. & Kvist, L. (2018). Bottlenecks, remoteness and admixture shape genetic variation in island populations of Atlantic and Mediterranean common kestrels *Falco tinnunculus*. *Journal of Avian Biology*, 49: e01768.
- Kvist, L., Broggi, J., Illera, J.C. & Koivula, K. (2005). Colonisation and diversification of the blue tits (*Parus caeruleus teneriffae*-group) in the Canary Islands. *Molecular Phylogenetics and Evolution*, 34: 501-511.
- Librado, P. & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25: 1451-1452.
- Lohman, D.J., Prawiradilaga, D.M. & Meier, R. (2009). Improved COI barcoding primers for Southeast Asian perching birds (Aves: Passeriformes). *Molecular Ecology Resources*, 9: 37-40.
- Martín, A. & Lorenzo, J.A. (2001). *Aves del archipiélago canario*. Lemus Editor. La Laguna.
- Mori, A., Giunchi, D., Rodríguez-Godoy, F., Grasso, R., Baldaccini, N.E. & Baratti, M. (2017). Multilocus approach reveals an incipient differentiation process in the Stone-curlew, *Burhinus oediconemus* around the Mediterranean basin. *Conservation Genetics*, 18: 197-209.
- Naguib, M., Hammerschmidt, K. & Wirth, J. (2001). Microgeographic variation, habitat effects and individual signature cues in calls of chiffchaffs *Phylloscopus collybita canariensis*. *Ethology*, 107: 341-355.
- Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E. & Wagner, H. (2018). *vegan: Community Ecology Package. R package version 2.5-3*. <https://CRAN.R-project.org/package=vegan>
- Päckert, M., Dietzen, C., Martens, J., Wink, M. & Kvist, L. (2006). Radiation of Atlantic goldcrests *Regulus regulus* spp.: evidence of a new taxon from the Canary Islands. *Journal of Avian Biology*, 37: 364-380.
- Padilla, D.P., Spurgin, L.G., Fairfield, E., Illera, J.C. & Richardson, D.S. (2015). Population history, gene flow and bottlenecks in island populations of a secondary seed disperser, the southern grey shrike (*Lanius meridionalis koenigi*). *Ecology and Evolution*, 5: 36-45.

- Pestano, J., Brown, R.P., Rodríguez, F. & Moreno, A. (2000). Mitochondrial DNA control region diversity in the endangered blue chaffinch, *Fringilla teydea*. *Molecular Ecology*, 9: 1421-1425.
- Raković, M., Neto, J.M., Lopes, R.J., Koblik, E.A., Fadeev, I.V., Lohman, Y.V., *et al.* (2019). Geographic patterns of mtDNA and Z-linked sequence variation in the Common Chiffchaff and the 'chiffchaff complex'. *PLoS ONE*, 14: e0210268.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, 67: 901-904.
- R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL <https://www.R-project.org/>
- Rousset, F. (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, 145: 1219-1228.
- Santos, A.M., Cabezas, M.P., Tavares, A.I., Xavier, R. & Branco, M. (2016). tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics*, 32: 627-628.
- Senfeld, T., Shannon, T.J., Grouw, H., Pajmans, D.M., Tavares, E.S., Baker, A.J., Lees, A.C. & Collinson, J.M. (2020). Taxonomic status of the extinct Canary Islands Oystercatcher *Haematopus meadewaldoi*. *Ibis*: doi:10.1111/ibi.12778.
- Spurgin, L.G., Illera, J.C., Jorgensen, T.H., Dawson, D.A. & Richardson, D.S. (2014). Genetic and phenotypic divergence in an island bird: isolation by distance, by colonization or by adaptation? *Molecular Ecology*, 23: 1028-1039.
- Stervander, M., Illera, J.C., Kvist, L., Barbosa, P., Keehnen, N.P., Pruijscher, P., Bensch, S. & Hansson, B. (2015). Disentangling the complex evolutionary history of the Western Palearctic blue tits (*Cyanistes* spp.) – phylogenomic analyses suggest radiation by multiple colonization events and subsequent isolation. *Molecular Ecology*, 24: 2477-2494
- Suárez, N.M., Betancor, E., Klasert, T.E., Almeida, T., Hernández, M. & Pestano, J.J. (2009). Phylogeography and genetic structure of the Canarian common chaffinch (*Fringilla coelebs*) inferred with mtDNA and microsatellite loci. *Molecular Phylogenetics and Evolution*, 53: 556-564.
- Tietze, D.T., Martens, J., Fischer, B.S., Y-Sun, H., Klussmann-Kolb, A. & Päckert, M. (2015). Evolution of leaf warbler songs (Aves: Phylloscopidae). *Ecology and Evolution*, 5: 781-798.
- Valente, L., Illera, J.C., Havenstein, K., Pallien, T., Etienne, R.S. & Tiedemann, R. (2017). Macroevolutionary dynamics in Atlantic island avifaunas support MacArthur and Wilson's equilibrium prediction. *Current Biology*, 27: 1660-1666.
- Valente, L., Phillimore, A.B., Melo, M., Warren, B.H., Clegg, S.M., Havenstein, K., Tiedemann, R., Illera, J.C., Thébaud, C., Aschenbach, T. & Etienne, R.S. (2020). A simple dynamic model explains island bird diversity worldwide. *Nature*, 579: 92-96.
- Van Doren, B.M., Campagna, L., Helm, B., Illera, J.C., Lovette, I.J. & Liedvogel, M. (2017). Correlated patterns of genetic diversity and differentiation across an avian family. *Molecular Ecology*, 26: 3982-3997.
- Weigelt, P., Steinbauer, M.J., Sarmiento Cabral, J. & Kreft, H. (2016). Late Quaternary climate change shapes island biodiversity. *Nature*, 532: 99-102.

SUPPLEMENTARY ELECTRONIC MATERIAL

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APPENDIX 1

Figure A1. Parsimony network of the Canary Islands Chiffchaff *Phylloscopus canariensis* performed with the TCS programme, based on COI sequences.

Figure A2. Bayesian consensus radial tree for the Canary Islands Chiffchaff *Phylloscopus canariensis* inferred with Mr. Bayes, based on cyt-b sequences.

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