

Population history of Berthelot's pipit: colonization, gene flow and morphological divergence in Macaronesia

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Abstract

The fauna of oceanic islands provide exceptional models with which to examine patterns of dispersal, isolation and diversification, from incipient speciation to species level radiations. Here, we investigate recent differentiation and microevolutionary change in Berthelot's pipit (*Anthus berthelotii*), an endemic bird species inhabiting three Atlantic archipelagos. Mitochondrial DNA sequence data and microsatellite markers were used to deduce probable colonization pathway, genetic differentiation, and gene flow among the 12 island populations. Phenotypic differentiation was investigated based on eight biologically important morphological traits. We found little mitochondrial DNA variability, with only one and four haplotypes for the control region and cytochrome *b*, respectively. However, microsatellite data indicated moderate population differentiation ($F_{ST} = 0.069$) between the three archipelagos that were identified as genetically distinct units with limited gene flow. Both results, combined with the estimated time of divergence (2.5 millions years ago) from the *Anthus campestris* (the sister species), suggest that this species has only recently dispersed throughout these islands. The genetic relationships, patterns of allelic richness and exclusive alleles among populations suggest the species originally colonized the Canary Islands and only later spread from there to the Madeiran archipelago and Selvagen Islands. Differentiation has also occurred within archipelagos, although to a lesser degree. Gene flow was observed more among the eastern and central islands of the Canaries than between these and the western islands or the Madeiran Islands. Morphological differences were also more important between than within archipelagos. Concordance between morphological and genetic differentiation provided ambiguous results suggesting that genetic drift alone was not sufficient to explain phenotypic differentiation. The observed genetic and morphological differences may therefore be the result of differing patterns of selection pressures between populations, with Berthelot's pipit undergoing a process of incipient differentiation.

Keywords: *Anthus berthelotii*, gene flow, oceanic islands, population genetics, recent dispersal, speciation

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Introduction

Island archipelagos, with their geographically discrete units supporting a range of differing habitats, environmental conditions and endemic species, have provided excellent study systems in which to investigate the phenomena of evolutionary radiation (Grant 1998; Whittaker 1998). The fauna of isolated oceanic islands also provide exceptional examples with which to examine the dispersal abilities of

different taxa, and for obtaining insights into rates of species diversification with time (Emerson 2002; Ricklefs & Bermingham 2007). The majority of studies of oceanic birds are macroevolutionary, utilizing mitochondrial DNA (mtDNA) markers that are very useful when populations have been isolated for some time (e.g. Warren *et al.* 2003, 2006; Filardi & Moyle 2005). Studies of less differentiated populations within widely distributed single species, incorporating nuclear markers and morphological variation are fewer in number. However, it is precisely these kinds of studies that provide an understanding of microevolutionary processes occurring in island forms (Clegg *et al.* 2002a, b).

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The Atlantic archipelagos included within the Macaronesian region (i.e. Azores, Madeira, Selvagens, Canary Islands and Cape Verde) have become a recent focus for studies of colonization and species diversification (Juan *et al.* 2000; Emerson 2002, 2003). In spite of this, and the fact that these islands are regarded as an Endemic Bird Area (Stattersfield *et al.* 1998), relatively few studies have examined patterns of colonization and diversification within Macaronesian birds. Nevertheless, the few genetic studies that have been undertaken have suggested that the occurrence of evolutionary radiation within archipelagos could be higher than previously thought (Dietzen *et al.* 2003; Kvist *et al.* 2005; Päckert *et al.* 2006). Studies of fine-scale genetic structure, critical for understanding the process of incipient speciation, are even rarer (Hille *et al.* 2003).

Because of their geographical location and relatively recent volcanic origin, the Macaronesian islands are an excellent system in which to investigate the evolution and radiation of birds. How isolated each island and/or archipelago is, both from the mainland and from other islands, differs greatly. For example, Fuerteventura (in the Canary Islands) is less than 100 km away from Africa, while the Azores are more than 1300 km away from the Iberian Peninsula. The geological age of the islands also varies greatly, from 1 to 29 million years old (El Hierro in the Canaries and Selvagen Islands, respectively; Geldmacher *et al.* 2001; Carracedo & Day 2002). This temporal availability of new islands and habitats has provided different opportunities for colonization and movement between islands over time. Furthermore, periodical volcanic eruptions, massive land events and palaeoclimatic processes, such as Quaternary glaciations, have produced changes in the original distributions of organisms because of the extinction, or fragmentation, of populations (e.g. Emerson 2003; Illera *et al.* 2006). Such events have also provided new habitats for recolonization, leading to range expansion and secondary contact between former populations (Brown *et al.* 2006; Emerson *et al.* 2006). These geological events provide prior information for inferring the timing of colonization and dispersal events of taxa, thus providing the context in which to investigate genetic and morphological differentiation within and between islands.

Molecular studies carried out on single species of native birds within the Macaronesian islands have revealed strong genetic differentiation between islands or groups of islands (e.g. Pestano *et al.* 2000; Dietzen *et al.* 2003; Kvist *et al.* 2005; Päckert *et al.* 2006). These studies suggest that the strong differentiation among populations is explained by ancient colonization events followed by a limited gene flow between islands. Overall, these studies suggest that once birds settle on islands, open water presents an effective barrier for isolating populations, facilitating diversification and speciation processes with time.

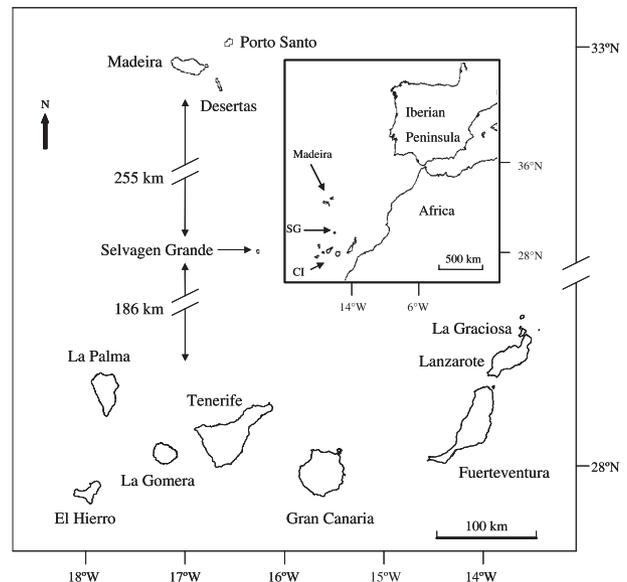


Fig. 1 The distribution of the Berthelot's pipit throughout the three Atlantic archipelagos. SG, Selvagen Grande; CI, Canary Islands.

Berthelot's pipit (*Anthus berthelotii*) is an ideal species in which to examine colonization patterns, dispersal abilities and diversification. It is a sedentary passerine endemic to the Madeiran archipelago, the Selvagens and the Canary Islands (Fig. 1), where it occurs on all islands and main islets (Martín & Lorenzo 2001; Oliveira & Menezes 2004). The pipit is both locally abundant (Martín & Lorenzo 2001; Illera *et al.* 2006) and widespread within islands (Martín & Lorenzo 2001). Berthelot's pipit has been suggested to have colonized the Macaronesian islands 2.5 million years ago (Voelker 1999a), although this is best considered as a maximum estimate as the phylogenetic data do not rule out a more recent colonization (see Emerson 2002). The species appears to have undergone some diversification within the archipelagos, with two subspecies recognized based on morphological differences – *Anthus berthelotii berthelotii* which occurs in the Canary and Selvagens Islands, and *Anthus berthelotii madeirensis* which is distributed throughout the Madeiran archipelago (Martín & Lorenzo 2001; Oliveira & Menezes 2004). Additional cryptic variation has been recorded within other species of the genus *Anthus* using molecular techniques (Voelker 1999b; references there in), and it is possible that substantial genetic divergence exists between the isolated populations of Berthelot's pipit. In addition to possible cryptic genetic divergence, Berthelot's pipit also provides an excellent opportunity to test the relative importance of random and selective processes as determinants of any, as yet unstudied, phenotypic differentiation within this species.

This study has three main aims. The first is to deduce the probable colonization pathway of Berthelot's pipit

across the Macaronesian archipelagos. The second is to quantify population differentiation and contemporary gene flow among the populations. To achieve these aims we use a combination of mtDNA and microsatellite DNA to resolve both broad-scale phylogeography, and fine-scale population structure. On the basis of the described differentiation into two subspecies, and apparent absence of movement between islands (Martín & Lorenzo 2001), we expect Berthelot's pipit to be undergoing incipient speciation throughout the three archipelagos it inhabits. Our third aim is to compare patterns of neutral genetic diversity (obtained with microsatellite markers) with variation in morphometric traits assumed to have evolved in response to both random genetic drift and different environmental conditions (Merilä & Crnokrak 2001; Willi *et al.* 2007). If random processes have determined morphological variation, we expect to find congruence between these and neutral genetic diversity; if this does not occur, then selective forces may be driving population differentiation (Clegg *et al.* 2002a).

Materials and methods

Study area, species, and field sampling

Berthelot's pipit is a small (16 g), sedentary, insectivorous passerine that breeds on all islands of the Madeiran archipelago and on the Selvagen and Canary Islands. These islands, located in the eastern North Atlantic, are separated by distances ranging from 1 to 589 km (Fig. 1). The pipit inhabits open, semi-arid habitats from sea level up to alpine habitats at elevations of 2500 m above sea level. Populations were sampled from each of the 12 main islands of the Madeiran archipelago (September 2006), Selvagens (April 2005) and Canary Islands (from January to March 2006).

Individuals were captured at multiple localities spanning the geography of each island in order to maximize the sampling of genetic variability within each island. Birds were captured using clap nets baited with *Tenebrio molitor* larvae and each individual was ringed with a unique numbered aluminium ring (Spanish Environmental Ministry). The age of all individuals caught (≥ 24 per island) was determined as either juvenile (Euring ages codes 3 or 5) or adult based on feather moult pattern (Cramp 1988) and eight morphometric traits were measured (see below). Blood samples (c. 40 μ L) were collected by brachial venipuncture, diluted in 800 μ L of 100% ethanol in a screw-cap microfuge tube and stored at room temperature. Birds were released at the point of capture.

Molecular procedures

DNA was extracted from blood using the standard salt-extraction method (Sunnucks & Hales 1996; Aljanabi & Martinez 1997) and diluted to a working concentration of

10–50 ng/ μ L. The sex of individuals was determined using the molecular methods set out in Griffiths *et al.* (1998).

To determine mtDNA variation, a 350-bp fragment of the Domain I of the control region was amplified using the primers H417 and L16743 (Tarr 1995). In many bird taxa, Domain I is the most variable of the three control region domains identified (Baker & Marshall 1997; Ruokonen & Kvist 2002) and, hence, the most informative for phylogeographical analyses. Additionally, a 941-bp fragment of the cytochrome *b* gene was amplified using primers L14841 (Kocher *et al.* 1989) and H16065 (Helm-Bychowski & Cracraft 1993) because some studies have showed that the control region is not always the most variable region of the mtDNA in birds (Zink & Blackwell 1998; Ruokonen & Kvist 2002). Polymerase chain reactions (PCR) were set up in 10- μ L total volumes including 5 μ L of 2 \times ReddyMix PCR Master Mix (ABgene), 0.5 μ L (10 mM) of each primer, 1 μ L MgCl₂ (25 mM) and 1.5 μ L of genomic DNA (25 ng/ μ L). PCRs were performed on a Tetrad 2 thermocycler with the following conditions: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, with an annealing temperature of 52 °C for 30 s, and extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. Sequencing reactions were performed using the PerkinElmer BigDye terminator reaction mix in a volume of 10 μ L using 1 μ L of PCR product and primers H417 (Tarr 1995), L14841 (Kocher *et al.* 1989) and H16065 (Helm-Bychowski & Cracraft 1993). The following conditions were used: initial denaturation at 94 °C for 2 min followed by 25 cycles of denaturation at 94 °C for 30 s, with an annealing temperature of 50 °C for 30 s, and extension at 60 °C for 2 min and a final extension at 60 °C for 1 min. The final product was sequenced on a PerkinElmer ABI PRISM 3700 automated sequencer.

All individuals were genotyped at five microsatellite loci that we had previously identified as being polymorphic in Berthelot's pipit: HRU5 (Primmer *et al.* 1995); PCA7 (Dawson *et al.* 2000); PPI2 (Martínez *et al.* 1999); LOX8 (Piertney *et al.* 1998); PDO5 (Griffiths *et al.* 1999). PCRs were set up in 10- μ L total volumes including 5 μ L of 2 \times ReddyMix PCR Master Mix (ABgene), 0.5 μ L (10 mM) of each primer (except PDO5 where only 0.25 μ L of each primer was used), 1.5 μ L of genomic DNA (25 ng/ μ L). Reverse primers were labelled at the 5' end with a fluorescent dye (FAM or HEX). Forward primers (except for LOX8) were also PIG-tailed with a 7-bp sequence added to the 5' end to minimize the production of stutter bands during genotyping (Brownstein *et al.* 1996). PCRs were performed with the following conditions: initial denaturation at 92 °C for 3 min followed by 35 cycles of denaturation at 92 °C for 30 s, with an annealing temperature dependent upon the specific primer set from 50.4 to 56 °C for 30 s, and extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. PCR products were sized using an automatic ABI PRISM 3700 sequencer using the ROX-500 size standard and GENEMARKER software (version 1.4).

Microsatellite data analysis

Hardy–Weinberg equilibrium and linkage disequilibrium were tested for at each locus using GENEPOP (version 3.4; Raymond & Rousset 1995). Statistical significance levels were obtained after using a sequential Bonferroni correction for multiple comparisons ($P = 0.01$; Rice 1989).

Genetic diversity in each population and at each locus was quantified by calculating allelic richness and expected heterozygosity using FSTAT (version 2.9.3; Goudet 2002). The significance of pairwise differences in heterozygosity and allelic diversity between each population was explored with one-way ANOVA tests. Overall genetic differentiation was calculated using a global F_{ST} value for all populations in GENEPOP. Genic and genotypic differentiation for all populations was determined using GENEPOP with the following parameters: 10 000 dememorizations, 100 batches and 5000 iterations per batch. To test population differentiation among islands, pairwise F_{ST} values were calculated in ARLEQUIN version 3.01 (Excoffier *et al.* 2006). Genetic differentiation among archipelagos was tested with an analysis of molecular variance (AMOVA). The significance of differentiation was tested against 50 000 permutations. To analyse the effect of geographical distance on genetic distance (F_{ST}), the Mantel test in ARLEQUIN was used, which computes correlation between distance matrices by a permutation procedure (Mantel 1967; Smouse *et al.* 1986). Geographical distances (to the nearest kilometre) were obtained as the straight-line distance between the closest coasts using Google Earth (<http://earth.google.com/>).

STRUCTURE (version 2.0; Pritchard *et al.* 2000) was used to determine the level of genetic structure without using previous information on the origin of each individual, and to detect possible movements of individuals between islands. We used the admixture model and the option of correlated allele frequencies between populations, performing five independent iterations at each level of genetic clustering (K ; for $K = 1$ –10), with a burn-in length of 30 000 and 1 million repetitions. Results at each value of K were averaged. It has been recently shown by Evanno *et al.* (2005) that the estimated log-likelihood of data computed by STRUCTURE, used to detect the number of populations (K), often does not match the real number of clusters. Consequently, we used the ad-hoc statistic (ΔK) provided by Evanno *et al.* (2005), which is based on the rate of change in the log probability of data between successive K values, for calculating the most likely number of clusters.

Both founder effects and population bottlenecks can lead to a reduction in the number of alleles in a population. However, immediately after a bottleneck the number of alleles is predicted to be reduced faster than heterozygosity. Therefore, one way to detect a recent bottleneck is to test for an excess of heterozygosity within a population (Cornuet & Luikart 1996). To explore evidence for recent genetic bottle-

necks in our populations, we used BOTTLENECK (Cornuet & Luikart 1996; Piry *et al.* 1999) following the recommendation suggested by Piry *et al.* (1999) when using fewer than 20 loci, whereby differences are tested with the one-tailed Wilcoxon's signed-rank test. We also used the two-phase mutation model (TPM) with 95% single-step mutations, 5% multiple-step mutations and a variance among multiple steps of 12 and 5000 iterations (Piry *et al.* 1999). We also used a second method of detecting bottlenecks, implemented in the same software, based on observed deviations from an L-shaped allele frequency distribution (Cornuet & Luikart 1996).

Signatures of population expansion were examined using the k and g tests (Reich & Goldstein 1998; Reich *et al.* 1999) which both use the distribution of allele sizes for detecting such events. The KGTTESTS Excel macro program developed by Bilgin (2007) was used to compute both the P value of k using the one-tailed binomial distribution and the g statistic. The significance of the g value in the KGTTESTS in Reich *et al.* (1999) was assessed using a table of 0.05 significant level cut offs for a range of numbers of loci and samples sizes.

To infer genetic relationships among populations, genetic distances (D_A) between populations (Nei *et al.* 1983) were calculated using DISPAN (Ota 1993). An unrooted neighbour-joining tree was constructed from these pairwise distances and branch arrangements assessed with 1000 bootstrap replications.

Morphological analysis

All individuals were measured by the same person (J.C.I.) using a digital calliper (± 0.01 mm) or a ruler (± 0.5 mm), and weighed using a digital balance (± 0.01 g). The following measurements were taken: wing length (maximum chord); tarsus length (bent method); tail length; head length from rear of skull to tip of bill; bill to skull length; bill width, bill height (the last two measurements; placing one of the callipers just in the middle of the nostrils) and weight. Because some pipits inhabiting alpine habitats were bigger than birds living near the coast (personal observation), the allometric effect of overall size was controlled for using a multivariate analysis of covariance (MANCOVA; Scheiner 2001). Consequently, size variation was first obtained using the first component (PC1) of a principal component analysis (PCA) performed with tarsus, head length and weight variables, which are good indicators of bird size (Rising & Somers 1989; Freeman & Jackson 1990). This factor was then used as a covariate in the MANCOVA analysis, where the rest of measurements were included as dependent variables, and island as a fixed factor. F -statistics derived from Wilks' lambda were used and any significant MANCOVA effect was tested for using multivariate pairwise contrasts with sequential Bonferroni correction (Scheiner 2001). Variables were log transformed and all statistical analyses were performed using SPSS plus (version 14.0). Normality

Table 1 Distribution of cytochrome *b* haplotypes among pipit populations. Number of base pair of difference of haplotypes 2, 3 and 4 with respect the most common haplotype (haplotype 1) is shown in brackets

Haplotype	Selvagen	Canary Islands							Madeira			Total	
	SG	FV	TF	LZ	GO	GC	LP	HI	GR	MA	DE		PO
1	5	5	4	3	4	4	5	4	4	5	5	5	53
2 (3)	0	0	1	1	1	0	0	0	1	0	0	0	4
3 (2)	0	0	0	1	0	1	0	0	0	0	0	0	2
4 (1)	0	0	0	0	0	0	0	1	0	0	0	0	1

Total, number of individuals recorded with each haplotype. SG, Selvagen Grande; FV, Fuerteventura; TF, Tenerife; LZ, Lanzarote; GO, La Gomera; GC, Gran Canaria; LP, La Palma; HI, El Hierro; GR, La Graciosa; MA, Madeira; DE, Desertas (Deserta Grande and Ileu de Chao); PO, Porto Santo.

of the transformed data and homogeneity of variance were assessed using the Kolmogorov–Smirnov test and Levene's test, respectively (Sokal & Rolf 1995). All transformed traits conformed with assumptions of normality and homogeneity of variance ($P > 0.05$).

Morphological and genetic differentiation

At neutral markers, genetic differentiation with time is thought to be determined mainly by drift (Hartl & Clark 2007). If morphological differentiation is also determined by neutral mechanisms, a match between morphological and neutral genetic differentiation is expected (Clegg *et al.* 2002a). The mean values of log-transformed morphological data were used in each population to calculate the Euclidean pairwise distances between populations. A Mantel test was used to compare this to the matrix of pairwise F_{ST} values previously obtained. Mantel analysis was performed with R software (Oksanen *et al.* 2006; R Development Core Team 2006), and significance was tested for with 10 000 permutations.

A second test for concordance between morphological and genetic differentiation – based on allelic richness and expected heterozygosity – was also performed. Morphological variability within populations was tested using a multivariate Levene's test (Dennison & Baker 1991; Clegg *et al.* 2002a). Residual values for each morphological trait were used to calculate the deviation values for each individual according to the formula proposed by Dennison & Baker (1991). The mean deviation (D) for each population was used as a measure of total variance. Linear regressions of the mean deviation vs. two different measures of genetic differentiation, provided by (i) allelic richness, and (ii) expected heterozygosity, were then performed (Clegg *et al.* 2002a).

Results

A total of 365 pipits were aged, measured and blood sampled (see Appendix for results of morphological traits by island and sex).

Mitochondrial DNA data

Five individuals per island were sequenced for each of the two mtDNA markers (60 individuals per marker) and sequences were aligned by eye using BIOEDIT (version 7.01). Because only one control region and four cytochrome *b* haplotypes were found, no further analysis was performed. All four cytochrome *b* haplotypes were found in the Canary Islands but only one in Selvagen Grande and Madeiran archipelago. The only one haplotype shared between the three archipelagos was also the most common haplotype found in the Canaries. Variants from the most common haplotype were due to 3, 2 and 1 bp of difference. The distribution of each haplotype is shown in Table 1. The control region and cytochrome *b* sequences have been deposited in the National Center for Biotechnology Information (NCBI) gene bank database under the accession no. of EF540814 (control region) and EU047720–EU047723 (cytochrome *b*).

Microsatellite data

Of the five loci used, only one (LOX8) showed significant departure from Hardy–Weinberg equilibrium. Excluding this locus from the analyses did not significantly change our results; hence, we used all five loci throughout the analyses to maximize the statistical power of tests, except where otherwise stated. Tests performed to detect linkage disequilibrium were not significant.

The lowest number of alleles and allelic richness per locus was found in Selvagen Grande, while the highest was recorded in the Canary Islands (Table 2). However, differences among populations were not significant for either heterozygosity ($F_{11,48} = 0.23, P = 0.99$) or allelic richness ($F_{11,48} = 0.40, P = 0.94$). The 12 populations showed a moderate level of overall genetic differentiation ($F_{ST} = 0.069$) and both allelic and genotypic distribution showed highly significant differences among populations ($P < 0.0001$). Analysing the pairwise F_{ST} values, Selvagen Grande showed the highest level of genetic differentiation relative

Table 2 The allelic richness and heterozygosity of microsatellite loci and populations. The analysis is based on minimum sample size of 24 diploid individuals. Expected and observed heterozygosities per locus and population are shown in brackets

	Canary Islands										Madeira				Total
	SG	FV	TF	LZ	GO	GC	LP	HI	GR	MA	DE	PO			
LOX8	5.95 (0.74/0.61)	18.62 (0.86/0.42)	21.91 (0.89/0.42)	17.05 (0.78/0.56)	17.75 (0.90/0.26)	18.36 (0.82/0.26)	14.76 (0.65/0.25)	16.55 (0.88/0.22)	16.00 (0.88/0.62)	12.19 (0.79/0.19)	9.15 (0.71/0.23)	9.30 (0.77/0.22)	22.21		
PCA7	1.00 (0.00/0.00)	2.00 (0.16/0.18)	2.00 (0.16/0.18)	2.00 (0.19/0.21)	2.00 (0.15/0.16)	1.99 (0.12/0.12)	2.00 (0.31/0.25)	1.95 (0.06/0.06)	2.00 (0.30/0.29)	1.00 (0.00/0.00)	1.00 (0.00/0.00)	1.00 (0.00/0.00)	1.97		
PP12	6.94 (0.81/0.77)	12.13 (0.87/0.87)	11.76 (0.85/0.75)	10.32 (0.81/0.90)	10.85 (0.80/0.80)	13.09 (0.84/0.75)	13.09 (0.84/0.75)	7.71 (0.75/0.83)	10.00 (0.78/0.79)	7.44 (0.78/0.81)	9.21 (0.77/0.80)	6.32 (0.64/0.64)	13.34		
PDO5	1.00 (0.00/0.00)	4.64 (0.41/0.39)	7.09 (0.47/0.45)	5.82 (0.53/0.53)	4.99 (0.61/0.60)	4.93 (0.55/0.41)	5.85 (0.64/0.64)	5.73 (0.66/0.80)	6.00 (0.56/0.54)	3.98 (0.44/0.54)	2.77 (0.39/0.51)	3.94 (0.35/0.32)	5.92		
HRU5	2.00 (0.41/0.32)	2.00 (0.49/0.66)	2.00 (0.47/0.51)	2.75 (0.51/0.52)	2.00 (0.47/0.56)	2.00 (0.45/0.45)	2.00 (0.35/0.39)	2.00 (0.47/0.51)	3.00 (0.49/0.62)	2.00 (0.49/0.63)	2.00 (0.47/0.32)	2.00 (0.49/0.61)	2.13		
Total	17 (31)	43 (33)	51 (33)	42 (32)	43 (30)	42 (31)	40 (28)	36 (31)	37 (24)	28 (31)	26 (30)	24 (31)	98		

Total, total number of alleles per locus and population (number of individuals used per island is shown in brackets). SG, Selvagen Grande; FV, Fuerteventura; TF, Tenerife; LZ, Lanzarote; GO, La Gomera; GC, Gran Canaria; LP, La Palma; HI, El Hierro; GR, La Graciosa; MA, Madeira; DE, Desertas (Deserta Grande and Ileu de Chao); PO, Porto Santo.

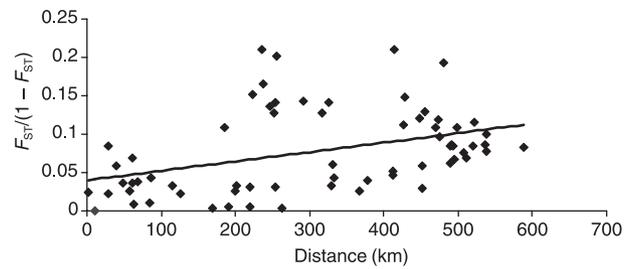


Fig. 2 Pairwise $F_{ST}/(1-F_{ST})$ values plotted against geographical distance (kilometre). Mantel test, $r = 0.42$, $P < 0.01$.

to the rest of the islands with all F_{ST} values of approximately 0.1 or above (Table 3). Of the 66 pairwise comparisons, only seven, based on comparisons between the eastern (Lanzarote, Fuerteventura and La Graciosa) and central (Tenerife and Gran Canaria) islands of the Canary archipelago (Fig. 1), resulted in nonsignificant F_{ST} values (Table 3). AMOVA results showed significant genetic variation among archipelagos ($F_{ST} = 0.097$, $P < 0.0001$), among populations within archipelagos ($F_{SC} = 0.037$, $P < 0.0001$) and within populations ($F_{CT} = 0.063$, $P < 0.0001$). Most variation was attributable to within-population variance (90.23%, $P < 0.0001$), but a small (and highly significant) amount of the variation was attributable among archipelagos (6.27%, $P < 0.0001$) and among populations within archipelagos (3.50%, $P < 0.0001$). The test for isolation by distance revealed a positive correlation between geographical distance and the genetic distance between populations, indicating genetic differentiation increases with increasing distances between islands (Fig. 2).

The initial genetic structure analysis identified a maximum of eight genetically distinct clusters. However, the use of the ad-hoc statistic (ΔK) resulted in a maximum of just three clusters (Fig. 3), corresponding to the three archipelagos. Most individuals from each of the Selvagens, Madeiran archipelago and Canary Islands (Table 4) were allocated to clusters I, II and III, respectively. Nevertheless, the lower proportion values of individuals assigned to each island in cluster III (Canaries) suggest a degree of gene flow among populations within cluster III (Canaries) and between cluster III (Canaries) and I (Selvagens). Likewise, a moderate proportion of individuals from Desertas (in the Madeiran archipelago) were assigned to cluster I (Selvagens) providing evidence that some gene flow occurs between this island as well (Table 4).

We did not detect a significant excess of heterozygotes in any of the populations (one-tailed Wilcoxon test: all islands $P > 0.8$, except Selvagen $P = 0.062$). Therefore, any reduction in allele number within a population was probably due to founder effects and not recent reductions in effective population size (N_e). Likewise, the distribution of allele frequencies was L-shaped, supporting the idea of

Table 3 Pairwise F_{ST} values with P values in brackets. Nonsignificant pairwise values were marked in bold

	SG	FV	TF	LZ	GO	GC	LP	HI	GR	MA	DE
FV	0.1213 (<0.001)										
TF	0.0944 (<0.001)	0.0067 (0.183)									
LZ	0.1424 (<0.001)	0.0018 (0.383)	0.0105 (0.074)								
GO	0.1273 (<0.001)	0.0328 (<0.001)	0.0210 (<0.01)	0.0330 (<0.001)							
GC	0.1336 (<0.001)	0.0133 (0.064)	0.0126 (0.075)	0.0045 (0.286)	0.0246 (<0.01)						
LP	0.1763 (<0.001)	0.0594 (<0.001)	0.0554 (<0.001)	0.0397 (<0.001)	0.0676 (<0.001)	0.0293 (<0.01)					
HI	0.1261 (<0.001)	0.0434 (<0.001)	0.0364 (<0.001)	0.0455 (<0.001)	0.0386 (<0.001)	0.0347 (<0.001)	0.0389 (<0.001)				
GR	0.1145 (<0.001)	0.0272 (<0.001)	0.0052 (0.292)	0.0251 (<0.01)	0.0277 (<0.01)	0.0322 (<0.01)	0.0511 (<0.001)	0.0311 (<0.001)			
MA	0.1284 (<0.001)	0.0636 (<0.001)	0.0511 (<0.001)	0.0771 (<0.001)	0.0595 (<0.001)	0.0723 (<0.001)	0.1337 (<0.001)	0.0731 (<0.001)	0.0629 (<0.001)		
DE	0.1156 (<0.001)	0.0809 (<0.001)	0.0743 (<0.001)	0.0996 (<0.001)	0.0824 (<0.001)	0.0944 (<0.001)	0.1653 (<0.001)	0.0790 (<0.001)	0.0813 (<0.001)	0.0558 (<0.001)	
PO	0.1696 (<0.001)	0.0895 (<0.001)	0.0942 (<0.001)	0.1155 (<0.001)	0.0100 (<0.001)	0.1085 (<0.001)	0.1763 (<0.001)	0.1045 (<0.001)	0.1094 (<0.001)	0.0775 (<0.001)	0.0376 (<0.01)

SG, Selvagen Grande; FV, Fuerteventura; TF, Tenerife; LZ, Lanzarote; GO, La Gomera; GC, Gran Canaria; LP, La Palma; HI, El Hierro; GR, La Graciosa; MA, Madeira; DE, Desertas; PO, Porto Santo

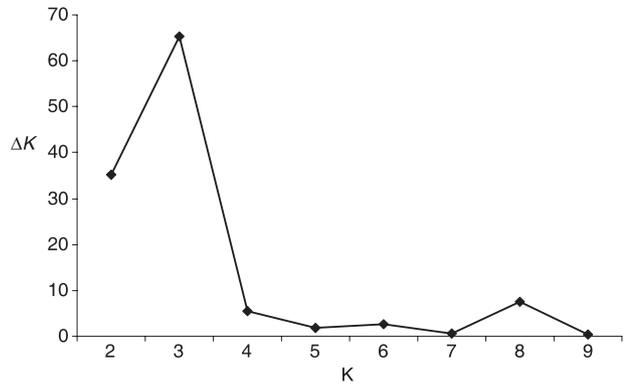


Fig. 3 Estimated modal values of ΔK (Evanno *et al.* 2005). The statistic ΔK calculates the most likely number of clusters. The highest height of the modal values of ΔK (reached at three clusters) corresponds with the uppermost level of structure.

Table 4 Proportion of individuals of each island assigned to each of the three clusters inferred without using prior population information in STRUCTURE

Islands sampled	Inferred clusters		
	I	II	III
Selvagen Grande	0.947	0.033	0.020
Fuerteventura	0.257	0.086	0.657
Tenerife	0.373	0.079	0.549
Lanzarote	0.304	0.080	0.616
La Gomera	0.403	0.136	0.461
Gran Canaria	0.235	0.167	0.597
La Palma	0.157	0.078	0.765
El Hierro	0.338	0.098	0.563
La Graciosa	0.469	0.078	0.454
Madeira	0.107	0.853	0.041
Desertas	0.425	0.528	0.047
Porto Santo	0.193	0.781	0.026

a long-term stable population size. Furthermore, the k test failed to reject the null hypothesis that population size has been constant, since the allele length distribution was not significantly different from a binomial distribution ($P = 0.16$). Finally, the g value ($g = 3.28$) did not support a history involving a population bottleneck and expansion.

The genetic relationships among populations are represented in Fig. 4. Islands within the Canary and Madeiran archipelagos cluster together, with high bootstrap support. Within archipelagos, there is moderate support for the clustering of some islands. In the Madeiran archipelago, Desertas and Porto Santo islands clustered together with the highest bootstrap value (73%) within any archipelago. Similar support (71%) was obtained for the clustering of El Hierro and La Palma in the Canaries.

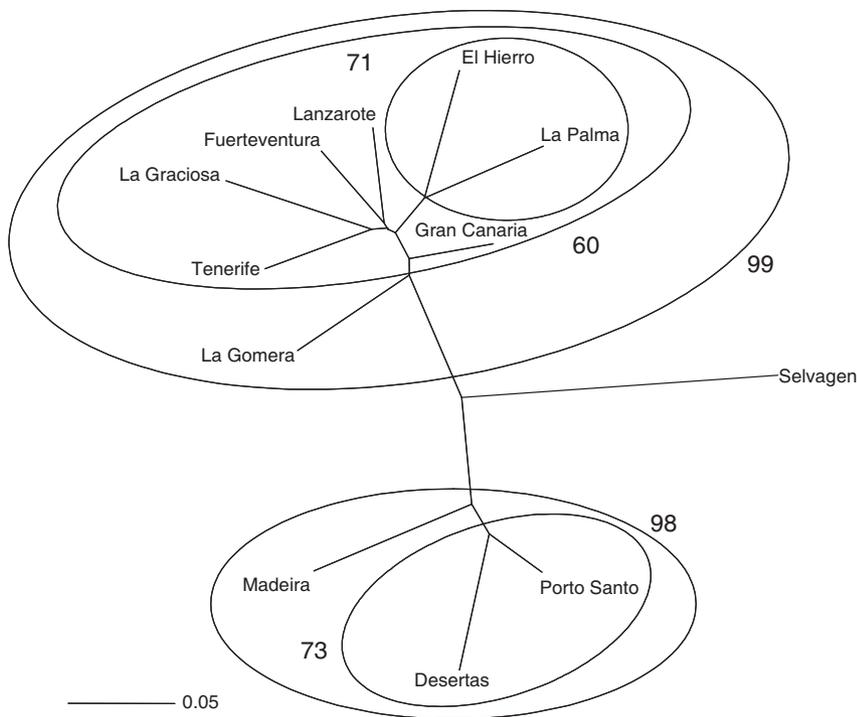


Fig. 4 Genetic relationships of Berthelot's pipit populations based on genetic distances (D_A) between populations (Nei *et al.* 1983). Numbers show bootstrap values (only values $\geq 60\%$ are shown).

Morphology

We found no morphometric differences among age groups for either sex (two-way ANOVAS, $P > 0.05$), but we did find differences between the sexes in weight, wing, tarsus and tail length ($F_{1,373} = 5.11$, $P = 0.024$; $F_{1,374} = 231.45$, $P < 0.001$; $F_{1,372} = 15.45$, $P < 0.001$; $F_{1,369} = 121.39$, $P < 0.001$, respectively). Because all results obtained for the different sex classes were similar, we will only show the results for males because of the higher sample size of this group (see Appendix), except where otherwise stated.

The MANCOVA identified significant morphological differences among islands ($F_{55,980} = 5.31$; $P < 0.001$). This overall difference was due to differences in wing length ($F_{11,215} = 5.33$; $P < 0.001$), bill length ($F_{11,215} = 9.38$; $P < 0.001$), bill width ($F_{11,215} = 6.26$; $P < 0.001$) and bill height ($F_{11,215} = 7.55$; $P < 0.001$) (see Appendix). Multivariate pairwise contrasts showed that wing length differences were explained by the difference between Selvagen Grande (the smallest population) and other islands ($P < 0.01$). Bill length differences were explained by differences between the Madeiran islands (the longest bills) and the islands of the other two archipelagos ($P < 0.01$). Bill width differences were due to differences between Selvagem Grande (the widest bill) and the Canary Islands ($P < 0.001$). Finally, bill height differences were due to differences between Selvagen Grande, Madeira Island and Desertas and some of the Canary Islands ($P < 0.05$).

Morphological and genetic differentiation

Pairwise F_{ST} and Euclidean distances were positively correlated with each other (Mantel statistic $r = 0.68$, $P < 0.01$). However, we failed to find a significant relationship between genetic and morphological indices of variation ($r^2 = 0.29$, $P = 0.07$; $r^2 = 0.23$, $P = 0.11$; for heterozygosity and allelic richness, respectively).

Discussion

Colonization and dispersal

Our results indicate that Berthelot's pipit has an unexpected pattern of colonization and diversification, differing from those previously inferred for endemic birds of the Macaronesian islands (Marshall & Baker 1999; Kvist *et al.* 2005; Päckert *et al.* 2006). Only one mitochondrial control region and four cytochrome *b* haplotypes were found throughout all pipit populations across the region, and indeed the majority of this variation only in the Canary Islands (the other islands were monomorphic at both mtDNA regions). One possible explanation for this pattern found could be that we had erroneously amplified a nuclear copy of the mtDNA fragment(s) (NUMT), which would evolve at a slower rate. However, we are confident that we are amplifying mtDNA for the following reasons. First, levels and patterns of similarity between our sequence

regions and those obtained from the meadow pipit (*Anthus pratensis*; Ödeen & Björklund 2003) and the tawny pipit (Arctander *et al.* 1996) were in accordance with those expected based on the rates of evolution observed in those regions. For example, for the control region, we found 93% similarity between the conserved domain II of the Berthelot's and meadow pipit; while at the cytochrome *b*, we found 96% similarity between Berthelot's and tawny pipit. Second, the two gene regions of mtDNA that we have used are genealogically congruent – consistent with their linkage within the mtDNA genome – but they are distantly separated physically and thus unlikely to be represented as a single NUMT.

Finding few mtDNA haplotypes throughout the range of Berthelot's pipit cannot be explained (exclusively) by high levels of gene flow as, by itself, this cannot dramatically reduce mtDNA variation. This fact also appears to be at odds with the idea that the evolutionary split between Berthelot's pipit and its sister species, the tawny pipit (*Anthus campestris*) occurred around 2.5 million years ago, after the dispersal of birds from the mainland to (and across) the Atlantic islands (Voelker 1999a). Although this divergence time may be an overestimate (Emerson 2002), it seems probable that both species diverged sufficiently long ago for greater mtDNA variability to be expected among Berthelot's pipit populations if all the islands were colonized at this point. The minimal mtDNA variability could be explained in one of two ways: (i) if the pipit has only recent dispersal across the region, or (ii) if mtDNA haplotype sharing across the region is the product of selection – the only mtDNA types that are observed are those that have survived a selective sweep. The implication of this second potential explanation is that pipits may have had greater mtDNA diversity in the past, but that this variation has been lost through natural selection. The presence of some mtDNA variation within the Canary Islands would seem at odds with such a scenario though. Additionally, with an mtDNA selective sweep, one would not necessarily expect a congruent pattern of marker variability at other, non-mtDNA, markers such as microsatellites, as is the case here. The alternative explanation of recent dispersal suggests that the low haplotype diversity we found is a consequence of a recent extension of the species range across the region. The presence of mtDNA haplotype diversity restricted to the Canary Islands suggests that the origin of this range expansion was the Canary Islands, and this is congruent with patterns observed for microsatellite allelic variation (Fig. 5) discussed below. Thus, it appears that the genetic population structure of the pipit is more consistent with a recent dispersal event from one initial source population within the archipelago, probably the Canary Islands, followed by limited gene flow among populations. This result is especially surprising as it contrasts with the few phylogeographical studies

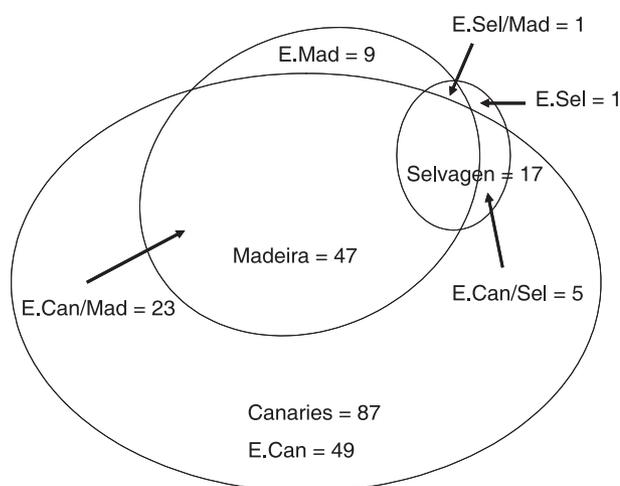


Fig. 5 Total number of alleles per archipelago. E.Mad, number of exclusive alleles in Madeiran archipelago; E.Sel, number of exclusive alleles in Selvagens; E.Sel/Mad, number of exclusive alleles shared in Madeiran archipelago and Selvagens.

published on Macaronesian birds, which suggest much older colonization and diversification events within the region (Marshall & Baker 1999; Kvist *et al.* 2005; Dietzen *et al.* 2006; Packert *et al.* 2006).

The low variability within the mtDNA meant that we were unable to definitively infer the colonization pathway of Berthelot's pipit across all the Atlantic islands. However, it was still possible to infer the sequence of dispersal between the three archipelagos using the distribution of both mtDNA haplotypes and nuclear microsatellite alleles. Both microsatellite allelic richness, number of alleles and exclusive alleles per locus was higher in the Canaries than in Selvagens Grande and in the Madeiran archipelago (Table 2 and Fig. 5). The pattern observed – alleles of Madeiran and Selvagens being subsets of those on the Canaries – could be explained either by a sampling artefact (i.e. small population size), or by the pipit having dispersed from the southern Canarian archipelago to the more northern island groups. In three of the five polymorphic loci studied (PCA7, PDO5 and HRU5), there were no alleles exclusive to Madeira and the Selvagens, all were subsets of those occurring on the Canaries. For the other two more polymorphic loci, only 11 alleles (nine for Madeira and one for the Selvagens, with another allele shared between the two) were exclusive to the northern island groups (Fig. 5). Assuming similar length mutation rate in all loci, it seems unlikely that only the Canaries would have undergone increased length variation for three microsatellite loci. More plausible is that the Canaries are the oldest inhabited archipelago, with most mutational variation originating there, and that later colonizers to the northern island groups possessed only a subset of these alleles. The

same pathway of dispersal can be deduced from the distribution of mtDNA haplotypes since Selvagen Grande and the Madeiran archipelago possess only one haplotype, shared with the genetically more variable Canary Islands.

The low mtDNA variability makes it impossible to estimate the timing of the pipits' dispersal across the Macaronesian region. Additionally, any attempt to relate dispersal time and some specific geological event would also be very speculative. However, it must have been very recent, more so than for other published studies of birds within the Macaronesian islands, as all these studies found higher mtDNA variability both between and within archipelagos (Marshall & Baker 1999; Pestano *et al.* 2000; Dietzen *et al.* 2003, 2006; Kvist *et al.* 2005; Päckert *et al.* 2006).

The inferred colonization pathway for Berthelot's pipit is contrary to the north to south pattern proposed for other Macaronesian land birds (Marshall & Baker 1999; Dietzen *et al.* 2003; Hille *et al.* 2003), which would be favoured by the prevailing northeastern or northwestern trade winds (but see Dietzen *et al.* 2006). However, other common phenomena, such as easterly winds blowing from Sahara, or strong southerly winds, are common during the winter, especially in the Atlantic archipelagos closest to the African mainland. Such climatic events could have facilitated the movement of birds from east to west and from south to north.

Population differentiation

Our results show a moderate but significant amount of genetic differentiation among pipit populations, which suggests a genetic substructure within and between archipelagos. As would be expected based on their geography, these differences were considerably stronger between, rather than within, archipelagos. Both the F_{ST} pairwise values (Table 3) and AMOVA tests suggest that restricted gene flow occurs, especially among the three archipelagos. A pattern that is supported by fact that three subpopulations, relating to the three archipelagos, were identified using the STRUCTURE program. This subdivision also corresponds to the pattern of isolation by distance revealed by the highly significant positive correlation between geographical and genetic distances. Within the archipelagos, the situation appears to be more complicated. The F_{ST} pairwise values suggest a moderate degree of gene flow occurs among the central and eastern islands of the Canaries, but lower gene flow between these islands and the rest of the Canary Islands (Tables 3 and 4). Furthermore, there seems to be little gene flow among the Madeiran Islands. The low overall genetic differentiation ($F_{ST} = 0.069$) recorded, in combination with the idea that some gene flow between the Canary Islands and Selvagens, and between the Selvagens and the Madeiran Islands (Table 4) may occur, could suggest that the statistical differences found may not reflect biological meaningful differences (Hedrick 1999,

2005). However, we failed to detect any factors, such as an excess of heterozygosity or bottleneck in any population (see below), which could have resulted in larger genetic distances between islands over a short period of time, and therefore given inaccurate estimates of divergence times between island (Hedrick 1999). Consequently, we are confident that our results reflect biological meaningful differences pertaining to a recent dispersal event. Therefore, both the microsatellite data and mtDNA variability clearly do not support the current division of the species into two subspecies, made based on bill morphology (Hartert 1910). Importantly, the microsatellite data provide clear evidence that limited gene flow and, consequently, genetic substructure of the metapopulation occurs among and within the archipelagos. In this context, we suggest that the pipit populations inhabiting the three Macaronesian archipelagos (i.e. Madeiran archipelago, Selvagens Islands and the Canary Islands) should be considered as three independent management units (Crandall *et al.* 2000).

Although we observed a reduction in allelic richness and number of alleles in Selvagen Grande and the Madeiran archipelago, we did not detect any evidence of a recent bottleneck/expansion in any of the island populations. It is possible that the pipit populations have, in fact, suffered bottlenecks but that we have failed to detect them. This may be the case if the events were not severe enough, in strength or duration, to cause a detectably high excess of heterozygosity (Nei *et al.* 1975; Leberg 1992). However, we also failed to detect significant differences in allelic diversity between populations, which provide a more sensitive indicator of changes in population size than heterozygosity excess (Nei *et al.* 1975). Therefore, our data suggest that the sequential colonization of the different Macaronesian islands by Berthelot's pipit was due to either several arrival events of medium size flocks, or by an arrival event of one flock of large size, such as has been recently demonstrated in *Zosterops lateralis* (Clegg *et al.* 2002b; Estoup & Clegg 2003). Both events would produce new and stable populations genetically representative of the original sources (Clegg *et al.* 2002b; Estoup & Clegg 2003).

The neighbour-joining tree used to infer genetic relationships between populations clearly separated the three archipelagos into three lineages (Fig. 4) consistent with the geography of the populations. Within archipelagos, the confidence with which populations could be clustered was low except for a cluster of the most western Canary Islands (71%), and the grouping of Desertas and Porto Santo cluster (73%) from the Madeiran archipelago.

Morphological differentiation

Phenotypic differentiation was found between pipit populations, although differences were mainly among archipelagos as opposed to among islands. However, examining differences

among archipelagos in detail reveals some irregular patterns, especially related to bill morphology. For instance, although Selvagen Grande was the smallest population in size, these individuals had the widest bill, and bill height was significantly higher in Selvagen than in all but two of the Canary Islands. Likewise, although individuals of the Madeira and Canary Islands were similar in overall body size, the three bill traits analysed were bigger in Madeiran individuals than in Canary specimens. These morphological trait differences may suggest that some microevolutionary processes are ongoing. What the selection pressures are, and how they differ between islands has not yet been explored. One possibility is that competitive interactions with other species may differ greatly between islands. Berthelot's pipit is an insectivorous bird that, on most islands, competes with other insectivorous species in the open habitats it feeds in (Martin & Lorenzo 2001). However, on the Selvagens, the pipit is the only breeding species of land bird (Oliveira & Menezes 2004) and, consequently the competition it faces is reduced. Studies on physiological adaptations, habitat selection, foraging behaviour, and competitive relationships are now needed, along with common-garden experiments, to understand the reasons behind the morphological differences recorded here (Scott *et al.* 2003).

Morphology and genetic differentiation

We have used two different approaches to test concordance between morphology and genetic differentiation in order to understand whether drift or selection processes are responsible for differences between populations. Our results were ambiguous. The significant concordance in the analysis of pairwise F_{ST} and Euclidean distances indicates that random processes are key. On the other hand, the absence of significant relationships between genetic and morphological indices of variation suggests that drift alone is not sufficient to explain phenotypic differentiation among populations (Clegg *et al.* 2002a). Overall, our results probably indicate that both processes are at work, although we could not quantify the effect of each of them on the morphological differences found. Consequently, it seems reasonable to suggest that the observed morphological differences may be the result of differing patterns of selection pressures between populations. Further analysis would be necessary to discriminate properly between both processes.

Conclusion

This study indicates that, contrary to previous thinking, Berthelot's pipit has only recently dispersed across the Macaronesian islands, and that the pattern of dispersal, from south to north, is opposite to that found for other

Macaronesian bird species. Berthelot's pipit shows little variability within the two mtDNA genes sampled, however, mtDNA patterns are consistent with the microsatellite data and suggest an origin in the Canary Islands. Analyses with microsatellite markers also indicate differentiation both between and within archipelagos, resulting in genetic structuring among the islands. Importantly, morphological differentiation could not be explained by drift alone. These results suggest that this endemic species, which diverged in the more distant past from its sister species, may provide an unexpected example of recent differentiation occurring across the Macaronesian islands. Such a system may be invaluable in determining the factors, patterns and processes that drive divergence and speciation.

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Appendix

Mean values (\pm SE) for morphological traits. The sample size is shown in brackets.

	SG		FV		TF		LZ		GO		GC	
	M	F	M	F	M	F	M	F	M	F	M	F
Wing	72.7 \pm 034 (27)	69.76 \pm 0.33 (25)	76.30 \pm 0.56 (10)	73.00 \pm 1.00 (2)	76.15 \pm 0.25 (24)	72.69 \pm 0.53 (8)	75.58 \pm 0.47 (12)	73.00 (1)	75.78 \pm 0.36 (23)	72.00 \pm 0.31 (7)	75.82 \pm 0.36 (22)	71.22 \pm 0.46 (9)
Tail	61.92 \pm 0.45 (26)	59.25 \pm 0.47 (22)	62.40 \pm 0.52 (10)	60.50 \pm 1.00 (2)	63.02 \pm 0.34 (24)	59.69 \pm 0.53 (8)	61.67 \pm 0.46 (12)	60.50 (1)	62.76 \pm 0.37 (23)	59.79 \pm 0.61 (7)	63.05 \pm 0.38 (22)	59.72 \pm 0.57 (9)
HeadL	32.66 \pm 0.16 (27)	32.69 \pm 0.16 (25)	33.37 \pm 0.13 (10)	32.99 \pm 0.04 (2)	33.48 \pm 0.11 (24)	33.42 \pm 0.04 (8)	33.83 \pm 0.15 (12)	32.16 (1)	33.67 \pm 0.11 (23)	33.04 \pm 0.18 (7)	33.85 \pm 0.13 (22)	33.36 \pm 0.23 (9)
Tarsus	21.58 \pm 0.10 (27)	21.51 \pm 0.11 (25)	22.09 \pm 0.19 (10)	21.80 \pm 0.08 (2)	22.66 \pm 0.12 (24)	22.27 \pm 0.21 (8)	22.64 \pm 0.17 (12)	21.54 (1)	22.37 \pm 0.11 (23)	21.90 \pm 0.22 (7)	22.29 \pm 0.13 (22)	22.13 \pm 0.13 (9)
BillL	15.32 \pm 0.12 (27)	15.24 \pm 0.13 (25)	15.67 \pm 0.14 (10)	15.29 \pm 0.17 (2)	15.68 \pm 0.10 (23)	15.37 \pm 0.36 (8)	15.82 \pm 0.15 (12)	14.72 (1)	15.50 \pm 0.08 (23)	15.15 \pm 0.11 (7)	15.78 \pm 0.10 (22)	15.47 \pm 0.13 (9)
BillW	3.64 \pm 0.03 (27)	3.69 \pm 0.04 (25)	3.45 \pm 0.04 (10)	3.42 \pm 0.02 (2)	3.47 \pm 0.03 (22)	3.60 \pm 0.05 (8)	3.49 \pm 0.03 (12)	3.59 (1)	3.50 \pm 0.03 (23)	3.54 \pm 0.01 (7)	3.49 \pm 0.02 (22)	3.50 \pm 0.03 (9)
BillH	3.25 \pm 0.03 (27)	3.24 \pm 0.03 (25)	3.08 \pm 0.03 (10)	3.02 \pm 0.14 (2)	3.18 \pm 0.02 (22)	3.12 \pm 0.03 (8)	3.17 \pm 0.03 (12)	3.10 (1)	3.15 \pm 0.03 (23)	3.08 \pm 0.03 (7)	3.11 \pm 0.02 (22)	3.09 \pm 0.02 (9)
Weight	16.44 \pm 0.22 (27)	15.84 \pm 0.29 (25)	16.01 \pm 0.15 (10)	17.35 \pm 0.55 (2)	16.17 \pm 0.19 (24)	16.39 \pm 0.34 (8)	16.21 \pm 0.14 (12)	15.80 (1)	16.28 \pm 0.15 (22)	16.59 \pm 0.63 (7)	16.21 \pm 0.16 (22)	16.64 \pm 0.46 (9)
	LP		HI		GR		MA		DE		PO	
	M	F	M	F	M	F	M	F	M	F	M	F
Wing	75.96 \pm 0.36 (23)	73.00 \pm 0.84 (5)	75.55 \pm 0.32 (20)	72.82 \pm 0.57 (11)	75.06 \pm 0.42 (18)	71.83 \pm 0.31 (6)	77.78 \pm 0.35 (23)	73.40 \pm 0.40 (10)	77.17 \pm 0.35 (18)	73.62 \pm 0.43 (13)	77.41 \pm 0.35 (17)	74.14 \pm 0.35 (14)
Tail	63.20 \pm 0.32 (23)	60.30 \pm 0.97 (5)	62.30 \pm 0.37 (20)	61.41 \pm 1.07 (11)	61.67 \pm 0.36 (18)	59.67 \pm 0.17 (6)	63.86 \pm 0.35 (22)	61.70 \pm 0.65 (10)	63.72 \pm 0.29 (18)	61.15 \pm 0.41 (13)	63.82 \pm 0.35 (17)	62.07 \pm 0.29 (14)
HeadL	33.53 \pm 0.07 (23)	33.07 \pm 0.25 (5)	33.33 \pm 0.12 (20)	33.01 \pm 0.15 (11)	33.53 \pm 0.12 (18)	33.15 \pm 0.20 (6)	35.17 \pm 0.11 (22)	34.62 \pm 0.16 (10)	35.44 \pm 0.14 (18)	35.20 \pm 0.09 (12)	34.92 \pm 0.08 (17)	34.57 \pm 0.14 (14)
Tarsus	22.48 \pm 0.10 (23)	22.00 \pm 0.28 (5)	22.84 \pm 0.10 (20)	22.61 \pm 0.14 (11)	22.41 \pm 0.12 (18)	22.40 \pm 0.25 (6)	22.95 \pm 0.12 (22)	22.52 \pm 0.18 (10)	22.71 \pm 0.11 (18)	22.75 \pm 0.15 (13)	22.84 \pm 0.13 (16)	22.31 \pm 0.16 (14)
BillL	15.53 \pm 0.07 (23)	15.32 \pm 0.24 (5)	15.62 \pm 0.09 (19)	15.21 \pm 0.10 (11)	15.47 \pm 0.08 (18)	15.52 \pm 0.07 (6)	16.86 \pm 0.12 (22)	16.58 \pm 0.09 (10)	17.02 \pm 0.12 (18)	17.02 \pm 0.08 (13)	16.61 \pm 0.08 (17)	16.44 \pm 0.08 (14)
BillW	3.48 \pm 0.01 (23)	3.51 \pm 0.03 (5)	3.48 \pm 0.02 (20)	3.47 \pm 0.04 (11)	3.46 \pm 0.02 (18)	3.50 \pm 0.03 (6)	3.58 \pm 0.02 (22)	3.59 \pm 0.03 (9)	3.57 \pm 0.02 (17)	3.58 \pm 0.03 (13)	3.59 \pm 0.04 (17)	3.56 \pm 0.02 (14)
BillH	3.09 \pm 0.02 (23)	3.06 \pm 0.03 (5)	3.13 \pm 0.03 (20)	3.15 \pm 0.04 (11)	3.07 \pm 0.02 (18)	3.04 \pm 0.04 (6)	3.30 \pm 0.02 (22)	3.25 \pm 0.03 (9)	3.27 \pm 0.02 (17)	3.18 \pm 0.02 (13)	3.18 \pm 0.03 (17)	3.16 \pm 0.02 (14)
Weight	16.42 \pm 0.15 (23)	16.28 \pm 0.21 (5)	16.32 \pm 0.15 (20)	15.44 \pm 0.29 (11)	16.18 \pm 0.13 (18)	16.72 \pm 0.88 (6)	17.63 \pm 0.18 (22)	16.77 \pm 0.20 (10)	17.06 \pm 0.24 (18)	16.15 \pm 0.24 (13)	17.15 \pm 0.19 (17)	16.41 \pm 0.16 (14)

SG, Selvagen Grande; FV, Fuerteventura; TF, Tenerife; LZ, Lanzarote; GO, La Gomera; GC, Gran Canaria; LP, La Palma; HI, El Hierro; GR, La Graciosa; MA, Madeira; DE, Desertas; PO, Porto Santo; M, male; F, female; Wing, length wing; HeadL, head length; BillL, bill to skull length; BillW, bill width; BillH, bill height.