

# A multiple trait assessment provides insights into the short-time viability of a newly founded population in an endangered island passerine

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#### Keywords

body condition; breeding success; Canary Islands; effectiveness of conservation actions; endangered island finch; genetic diversity and relatedness; global warming; translocation; upland forest passerine.

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# **Abstract**

Determining the effectiveness of conservation actions is a priority in conservation biology, especially in island ecosystems which can host large numbers of endemic and often threatened species. In this study, we have brought together a genetic, body condition and breeding success assessment with the aim of evaluating the viability of a newly founded population of the endangered Gran Canaria blue chaffinch (Fringilla polatzeki), the forest passerine species with the most restricted distribution in the Western Palearctic. The species occurs exclusively in the Canary pine forests (Pinus canariensis) of the island of Gran Canaria, with the Inagua nature reserve harbouring the highest number individuals. In 2010, a translocation program was initiated within the same island in the nearby pine forests of La Cumbre with the goal of establishing a viable breeding population. Genetic results revealed that La Cumbre shows genetic parameters (diversity, inbreeding, and relatedness) similar to the core source of Inagua, which contrasts with the reduced genetic diversity expected due to the small size of the newly founded population. The biometric and body condition results (wing and tarsus length, body mass, and length asymmetry of the tail feathers), together with the breeding success (nest survival and number of fledglings per successful breeding attempt), were also similar in both populations. Overall, these findings suggest that the translocation program has been successful and provide insights on the effectiveness of the actions performed. Our evaluation also delivers future avenues for the conservation planning in other upland forest endangered avian species inhabiting island ecosystems, especially those threatened by the effects of global warming.

# Introduction

Newly founded populations are the result of either the arrival and settlement of a group of individuals from nearby areas (e.g., island colonisations), or by the deliberate movement of specimens between areas (i.e., introductions and translocations; Komdeur, 1994; Clegg, 2010; Ricklefs, 2010). Translocation programs, from source populations or captive breeding, are important strategies for the conservation of highly threatened species. These programs seek to increase the genetic diversity of the target population, making it more resistant to environmental changes or diseases and enhancing the breeding output and fitness of target species. Ultimately, this approach can lead to the establishment of new viable populations (Allendorf et al., 2022). This is of particular

concern in the context of global warming and intense environmental transformation, where the remaining habitats for threatened species may not be in the range of their climatic preferences (Naujokaitis-Lewis, Endicott, & Guezen, 2021; Halupka *et al.*, 2023). Translocations can help species adapt to changing climate conditions by moving individuals to areas with more suitable habitat or environmental conditions (Fortini *et al.*, 2017). Such conservation actions will be more effective if they promote connectivity between the source population and translocated ones (Berger-Tal, Blumstein, & Swaisgood, 2020). Translocations often involve unreplicated ecological experiments, due to logistical challenges and associated costs, it is not feasible to replicate them across multiple locations (Morris *et al.*, 2021). Successful translocations are based on pre-existing knowledge of natural history of

species, and the availability of suitable locations for the endangered species considering the climatic conditions, safety, and habitat preferences (Berger-Tal, Blumstein, & Swaisgood, 2020: Jahn et al., 2022). Therefore, translocation programs need to be evaluated over time to know their effectiveness by collecting evidence on how translocated individuals have contributed to establishing new populations with high genetic variability, and viability to reproduce naturally (Pérez et al., 2012; Gerber et al., 2018; Berger-Tal. Blumstein, & Swaisgood, 2020; Resende et al., 2020; Sutherland et al., 2021). Nevertheless, with these limitations, translocation programs can provide valuable insights into the biology, ecology, and behaviour of species, informing future conservation efforts and contributing to our overall understanding of how to rescue endangered species from the brink of extinction.

Recently established populations typically occupy small patches, harbouring a low number of individuals. Theoretical population genetics predicts that small populations should experience reduced genetic diversity in comparison to bigger ones (Wright, 1938) and exhibit high demographic stochasticity and low-standing genetic variation (i.e., the genetic variants available for the action of the natural selection), factors that hamper their survival over time (Frankham, 1995; Jensen et al., 2007). Small isolated populations are expected to show stronger genetic drift effects with rapid loss of heterozygosis than bigger ones, which is a consequence of their reduced effective population sizes (Kimura, 1983; Frankham, 1997). Small populations are also expected to exhibit high inbreeding values and high rates of slightly disadvantageous mutations (Hoffmann, Sgrò, & Kristensen, 2017; Verena et al., 2020; Leroy et al., 2021; Jackson et al., 2022; Martin et al., 2023). The biological consequences are well known and include negative impacts on components of reproductive success, such as fertility, fecundity, variation in body size, and survival rates, which threaten the viability of these populations in the face of future environmental changes (Briskie & Mackintosh, 2004; Agudo et al., 2012; Niskanen et al., 2020; Duntsch et al., 2023).

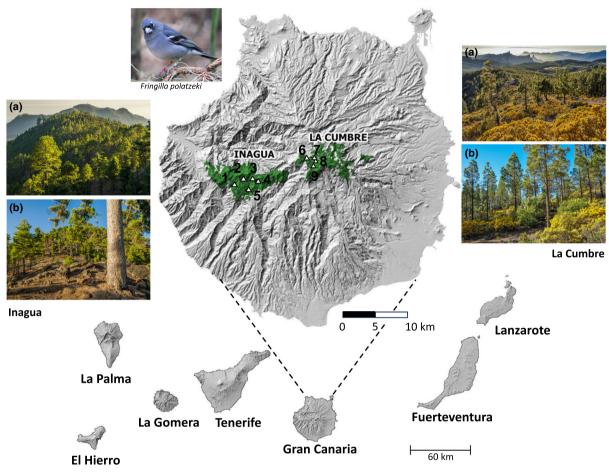
Unpredictable environmental events, such as extreme climatic conditions, fires, volcanic eruptions, together with anthropic actions, also enhance the chance of extinction for the small populations (Frankham *et al.*, 2017; Allendorf *et al.*, 2022). To understand the needs of a small population that has recently been established via translocation is one of the most critical challenges in conservation biology (Dennis, Munholland, & Scott, 1991; Mills, 2013; Horne *et al.*, 2016; Marrero *et al.*, 2019). Comprehensive studies that consider various interconnected aspects of a species' natural history and biology are a suitable strategy to assess the viability of populations, by gathering information on genetic structure, breeding success, habitat quality, trophic ecology and diseases that will inform as to the persistence of small populations over time.

The Gran Canaria blue chaffinch (*Fringilla polatzeki*) is an endemic avian species of the Canary Islands, with a very small geographic range and population size (<50 km<sup>2</sup> and <500 adult birds, respectively), listed by the IUCN as

"Endangered" (BirdLife International, 2016). For a long time it was thought to be the same species as the Tenerife blue chaffinch (Fringilla teydea), as an exclusive inhabitant of Canary Island pine forests (Pinus canariensis). Its taxonomic status changed in 2016 from subspecies to a clearly differentiated species, based on genetic distance and morphological and acoustic traits (Lifjeld et al., 2016; Sangster et al., 2016). While the Tenerife blue chaffinch is considered not threatened, with populations occurring throughout the large forests surrounding Teide peak, this is not the case with the blue chaffinch of Gran Canaria, where the pine forests are scarce and highly fragmented due to an intensive secular human management. As a consequence, the Gran Canaria blue chaffinch population is very small and has historically been restricted to only two forests on the island, the core population at the Integral Natural Reserve of Inagua, and a much smaller one in the pine forest of Tamadaba, from where it disappeared as a stable breeder in 1991 (Fig. 1; Martín & Lorenzo, 2001). The blue chaffinch density in Inagua has remained relatively stable at around 9-16 birds/km<sup>2</sup>, except after the two years immediately following the 2007 forest fire that reduced its population by half (Moreno et al., 2018). This is the lowest abundance recorded for a small forest passerine in the entire Western Palearctic (Keller et al., 2020). The very restricted distribution in Gran Canaria is a consequence of blue chaffinch's selection for mature, sparsely wooded pine forests (35-55% of canopy cover with pine height c. 19 m), located at altitudes of over 1200 m (Carrascal et al., 2017, 2022).

Since 2008, a small contingent of individuals of the Gran Canaria blue chaffinch has been established at La Cumbre, a small area (20.7 km<sup>2</sup>) of reforested Canary pine forest, located at a minimum distance of 2 km from the core area of Inagua. A few individuals, of unknown number but probably less than 6 birds, naturally colonised La Cumbre in 2008 and 2009 after the 2007 forest fire in the Inagua pine forests. A translocation program was initiated in 2010 in the pine forests of La Cumbre with the final aim of establishing a viable breeding population. The program originally began with individuals coming from a captive breeding centre located at Tafira (Delgado et al., 2016). This translocation program was reinforced through a LIFE Project starting in 2015, with more individuals released from the captive breeding centre and additional wild birds captured in the Inagua source population (LIFE+PINZÓN, 2019). By expanding the habitat range of the Gran Canaria blue chaffinch's sole remaining population in Inagua, the translocation program aims to mitigate the risks of localised environmental catastrophes, such as forest fires, which have historically devastated its limited and very small population. A total of 199 blue chaffinches were released at La Cumbre between 2010 and 2019, 116 born in captivity and 83 from the wild population of Inagua. Other pine forests on the island were discarded because of their very low suitability for the species, given the climatic conditions and vegetation structure (Carrascal et al., 2017).

The main goal of the present study was to assess the short-term viability of the newly founded population as a



**Figure 1** Study areas (green) in Gran Canaria Island (Canary Islands, Spain). Woodlands in La Cumbre are pine plantations (*Pinus canariensis*) mainly established after 1960 (a: general view; b: inside forest), while Inagua is an historic natural pine forest (a: general view; b: inside forest). White triangles indicate the sites where juvenile birds were captured for morphometric and genetic analyses. Numbers show the name of each site. 1 – Aula de la Naturaleza; 2 – Agujero; 3 – Alsandra; 4 – Palmera; 5 – Mulato; 6 – Nublo; 7 – Francés; 8 – Los Hornos; 9 – Bailico. Photos: A. Delgado (Gran Canaria Blue Chaffinch); L.M. Carrascal (pine forest pictures).

result of the translocation program carried out with the endemic Gran Canaria blue chaffinch during the past 12 years (2010-2021). To achieve this objective, we compared the daily temperature as well as various biotic traits, including genetic, morphological, breeding success, and body condition between La Cumbre (the newly founded) and Inagua populations. Global warming can affect the phenology of avian species, for instance, advancing mean laying dates (Andreasson, Nord, & Nilsson, 2023) but the intra-specific response could be dissimilar across species' range distribution (Bailey et al., 2022). Environmental features related to rainfall and solar radiation have been pointed important to explain the successful nesting and the spatial variation in density of the blue chaffinch in Inagua (Carrascal et al., 2017, 2022). Therefore, it is pertinent to assess differences in temperature regimen between the core population in Inagua and the translocated population in La Cumbre during the breeding season. This comparison aids in understanding the environmental suitability of the new area, especially in

light of projected temperature increases and more frequent heatwaves. Data on the genetic diversity (i.e., amount of variation observed among different individuals), and other genetic parameters such as effective population size (N<sub>e</sub>), relatedness and inbreeding, between the source and the translocated population will provide information about the potential long-term survival and adaptability of La Cumbre. We know that these genetic parameters affect the ability of individuals to respond to environmental changes and resist diseases (Bonneaud et al., 2011; Gonzalez-Quevedo et al., 2016). Thus, similar genetic values between these populations would support the survival and adaptability of La Cumbre individuals in the short time of 12 years after the beginning of the translocation program. On the other hand, fluctuating asymmetry (FA) represents the random deviations from perfect bilateral symmetry in organisms, which are commonly considered to be a consequence of developmental instability in the face of environmental and genetic stressors (Palmer & Strobeck, 1986; Møller & Swaddle, 1998). FA offers insights into the overall body condition during growth due to its sensitivity to environmental stress and response to differences in phenotypic quality (Clarke, 1992, 1995; Polak & Trivers, 1994). In a similar way that we stated with the genetic trait, comparable FA values between both populations would suggest that birds from La Cumbre are not experiencing higher environmental stress than Inagua. Such a result would support that extant individuals of La Cumbre show suitable phenotypic conditions to persist in the short time. Finally, data on breeding success, biometry and body condition of birds hatched the previous spring will indicate whether there were any major constraints during the breeding period and subsequent emancipation of the fledglings between both sites. Overall, a comparison of genetic diversity and inbreeding levels, effective population size and genetic differentiation, as well as body condition, breeding success and variation in juvenile body size or mass between the Inagua and the La Cumbre populations will provide meaningful insights into the effectiveness of the conservation actions initiated in 2010.

#### **Materials and methods**

# **Species**

The Gran Canaria blue chaffinch is a small (~27 g) passerine occurring in the Canary Island pine forests of Gran Canaria. The blue chaffinch has a robust beak capable of opening the Canary pine cones on which seeds it feeds (Grant, 1979). Its diet also includes other seeds and invertebrates, the latter being the main food of the chicks (Martín & Lorenzo, 2001). The breeding season occurs mainly between mid-May and mid-July, when females lay one or two clutches consisting of two eggs (approximately only one third of the females produce two clutches per year; Rodríguez & Moreno, 2008). Juveniles of any breeding season will breed in the next breeding period. The very low clutch size is surprising when compared to other European and African Fringilla finches (mostly 3 to 7 eggs per clutch; Perrins, 1998). However, this low clutch size can be seen as an "island syndrome". This phenomenon could be explained by the reduced predation pressure on the islands, and the less challenging environment in the Canary Islands, particularly during winter, factors that would influence blue chaffinch survival (Lack, 1947; Cody, 1966; Covas, 2011). The species has few potential natural predators: Eurasian sparrowhawk (Accipiter nisus), Longeared owl (Asio otus), and Great spotted woodpecker (Dendrocopos major), and Raven (Corvus corax) as nest predator (Rodríguez & Moreno, 2004). The population size in Inagua was stable until the severe fire of 2007 where the population density decreased by half (Moreno et al., 2018). However, since 2008, the population has significantly increased, and in 2019, it reached the highest values recorded for this species with 362 birds (257-489, 95% CI; Carrascal et al., 2022). Finally, according to the colour banding program established more than 30 years ago, the species moves out the territories after ending the breeding season. These movements were within Inagua, being rare dispersal events among nearby

localities (Martín & Lorenzo, 2001). A very small proportion of wild birds from Inagua (<6%) regularly visited La Cumbre; conversely, 25–58% of the wild blue chaffinches that were colour-banded in La Cumbre regularly visited the Inagua pine forests during the breeding season (Delgado *et al.*, 2020). Most of the individuals (92%) of the translocation program were juveniles (EURING age code 3) and were released in the last week of September or the first week of October from 2010 to 2018 (LIFE+PINZÓN, 2019).

#### Study areas

The main study area (hereafter La Cumbre) is located in central highest part of Gran Canaria (27° 58' N, 15° 35' W), an island of volcanic origin (1560 km<sup>2</sup>). Altitudes in this area range from 1070 to 1950 m above sea level (a.s.l.) (see González, Rodrigo, & Suárez, 1986; Santos, Morales, & Pérez-González, 2000, for details on the vegetation cover and landscape characteristics). It covers 20.7 km<sup>2</sup> of pine plantations of Pinus canariensis, with some scattered shrublands (Teline microphylla, Adenocarpus foliolosus and Chamaecytisus proliferus) and yards, mainly above 1180 m a.s.l. The other study area for comparison is Inagua Integral Natural Reserve (37.59 km<sup>2</sup>), a natural, historic, pine forest of *Pinus* canariensis, spanning 850-1575 m a.s.l., which harbours the main extant breeding population of the blue chaffinch known since the beginning of twentieth century (Martín & Lorenzo, 2001). In this comparative context, the population of Inagua is an ideal reference point because is the only remaining population, and it has shown striking resistance and resilience after 2007 as a result of a catastrophic fire that reduced its numbers by half (Suárez et al., 2012; Moreno et al., 2018). Detailed information about these areas and the blue chaffinch populations can be found in Rodríguez & Moreno (2008), Carrascal et al. (2017, 2022) and Moreno et al. (2018). The geographical location of La Cumbre and Inagua study areas is shown in Fig. 1.

# **Environmental procedures and data analysis**

There are marked differences in the vegetation structure of the pine forests between Inagua Strict Nature Reserve (I) and La Cumbre (LC) (Carrascal *et al.*, 2016). Thus, LC showed higher pine and shrub cover than I, although with lower pine average height (Table 1). Altitude was, on average, higher in La Cumbre than in Inagua (Table 1).

Precise data on air temperature in the areas of Inagua and La Cumbre, where the blue chaffinch was more abundant during the breeding season 2017, were obtained by means of four temperature loggers in La Cumbre and eight in Inagua (Onset HOBO Pendant). Loggers were placed in the shade on thick trunks, oriented to the north and at approximately 1.5 m above ground. Data loggers recorded air temperature every 15 min from 30th May to 30th September. For each recording day (96 measurements), minimum, maximum, average daily temperature and amount of time with air temperature higher than 30°C were obtained (Table 1).

Table 1 Habitat and temperature characteristics for the two pine forests where the Gran Canaria blue chaffinch occurs

	Inagua	La Cumbre
Pine cover (%)	26.5 (13.2)	46.1 (24.4)
Pine height (m)	17.1 (5.7)	13.5 (4.2)
Shrub cover (%)	8.7 (9.9)	18.3 (16.0)
Altitude (m)	1121 (163)	1591 (194)
Hobo altitude (m)	1326 (1231–	1660 (1504–
	1465)	1781)
Minimum temperature (°C)	18.3 (0.48)	16.7 (0.37)
Maximum temperature (°C)	34.4 (0.51)	32.5 (0.45)
Average temperature (°C)	23.8 (0.48)	21.9 (0.38)
Minutes/day temperature >30°C	269.6 (23.1)	183.4 (17.4)
Maximum absolute temperature (°C)	43.3	40.6

Values represent mean values and standard errors (in brackets). Hobo altitude: Average altitude and range (in brackets) of Hobo locations in La Cumbre and in Inagua.

Temperature data for the eight data loggers in Inagua and four data loggers in La Cumbre were averaged for each one of the 124 days from 30th May to 30th September. The relationships between temperature data for La Cumbre and Inagua were established considering the existence of time autocorrelation, using GLS (Generalised Least Squares) models with ARMA (AutoRegressive –p– Moving Average –q–) autocorrelation terms. Different models combining autoregressive p figures and moving average q figures from 1 to 3 (nine models) were built, and the model with the lowest AICc was selected. Packages {nlme} (Pinheiro *et al.*, 2022) and {MuMIn} (Bartoń, 2022) were used under R analytical environment (R version 4.2.0; R Core Team, 2022).

#### Field sampling and morphological analysis

Juvenile birds hatched in the previous spring were captured during the second fortnight of August and the entire month of September of five consecutive years (2017–2021). In total, we caught 125 birds for biometric analyses. They had the right fifth and left fifth tail feathers (rectrices) removed to estimate fluctuating asymmetry, and to obtain DNA from feathers. The day of capture of juvenile blue chaffinches in late summer did not differ significantly between Inagua and La Cumbre (P = 0.464; date 1.6 days later in Inagua). Therefore, the biometric differences between the juvenile birds of the two populations cannot be associated with differences in the dates of their capture.

We captured birds using mist nets in five sites of Inagua (El Mulato, Alsándara, Aula de la Naturaleza, El Agujero, La Palmera), and four sites in La Cumbre (Los Hornos, Bailico, El Francés, Nublo), separated by a distance of c. 11 km (Fig. 1). We ringed the individuals with a unique combination of a numbered metal, and two-coloured, rings. We sexed (male/female) and aged (juvenile/adult) all birds based on colour and feather moult pattern (Cramp & Perrins, 1994; Shirihai & Svensson, 2018), and four morphometric traits were measured: tarsus-metatarsus length (±0.01 mm) with a

digital calliper, tail length and wing chord (±0.5 mm) with a ruler, and body mass with an electronic balance ( $\pm 0.05$  g). Before releasing birds in the same capture site, we plucked the right and left fifth tail feathers (rectrices). We checked that rectrix growth had stopped by observing the absence of blood in the part of the shaft within the follicle and feather sheaths (Ginn & Melville, 1983). The full length of each feather, laid perfectly flat and stretched, was measured twice on different occasions using a ruler (+0.5 mm). Deteriorated feathers at the tip were discarded because their length could not be accurately measured. The absolute difference between the right and left tail feathers of each animal was used as a measure of growth asymmetry. This difference was expressed as the percentage of the average length of both feathers. Repeatability (see Lessells & Boag, 1987) of the length of left and right feathers of the studied 125 birds was very high (0.998; using the sum of squares of a one-way ANOVA with a factor coding for the different individual birds).

Two-way ANOVAs of tarsus, wing and tail length were applied in order to test the statistical differences between sexes (females vs. males) and study areas (Inagua vs. La Cumbre) and their interaction. A two-way ANCOVA was applied to the analysis of log-transformed body mass, including tarsus length as a covariate. The two-way ANOVA of tail feather asymmetry was carried out using the Box-Cox transformation (Sakia, 1992;  $Y' = [Y^{\lambda} - 1]/\lambda$ ).

#### **Breeding success**

Intensive surveys from April to August of La Cumbre pine forests during 2017 to 2021, allowed the location of active nests (all sampling carried out by A.D, with a prospection intensity of around 75 days-person per year). The method for the location and monitoring of blue chaffinch nests has been previously described in detail by Rodríguez & Moreno (2008) and Carrascal et al. (2017). Nests were monitored every 3-5 days in order to establish the successful reproduction and the amount of chicks fledged. We considered a successful breeding attempt when at least one fledgling was produced in the focal nest. Due to the low proportion of second broods in the population (33% according to Rodríguez & Moreno, 2008), some logistic difficulties regarding the fact that breeding by some pairs can be prolonged until the first week of August, we could not obtain reliable data on second broods. Some nests were disregarded in data analyses due to our inability of ascertain if they were first or second broods considering the late laying date for first broods at the end of June and the beginning of July.

To account for nest-failure detection, we analysed nest survival using the MARK software (v.8; White & Burnham, 1999, Dinsmore, White, & Knopf, 2002). MARK uses a maximum likelihood estimator and a logit link-function to estimate the probability of daily nest survival (DSR) based on the days of exposure (Mayfield, 1961). Cumulative estimate of nesting success was calculated raising DSR to the number of days of the nest cycle (*i.e.*, mean period of eggs and chicks that equals 32 days in the case of Gran Canaria blue chaffinch). The delta method was used to calculate the

variance of nesting success estimates (Powell, 2007). Nest survival at Inagua was estimated by Rodríguez & Moreno (2008) using Mayfield's estimator, which assumes that nest survival is constant over time. This value was compared with the constant model of Nest Survival at La Cumbre obtained in MARK. Confidence intervals for the number of fledglings and the probability of having two fledglings per successful breeding attempt were obtained by bootstrapping the data (20 000 iterations).

#### Molecular procedures and data analyses

In total, we extracted DNA from 107 Gran Canaria blue chaffinches from the feathers using the ammonium acetate method (Illera, 2023): 57 birds caught at the core population of Inagua, and 50 in the recently founded population at La Cumbre (Fig. 1; Table S1).

We genotyped all individuals for nine microsatellite polymorphic loci, which have previously been designed to assist in Gran Canaria blue chaffinch management (Suárez et al., 2009). We arranged the polymerase chain reactions (PCRs) into two multiplex reactions obtained with the program Multiplex Manager version 1.0 (Holleley & Geerts, 2009). The two multiplex panels included four (Ftey19; Ftey22; Ftey26; Ftey29) and five (Ftey08; Ftey20, ftey25; Ftey28; Ftey30) pairs of primers with 6-FAM and HEX fluorescent dye-labelled forward primers. PCRs were set up in 4 µL volume including 2 µL de 2x QIA-GEN Multiplex PCR Master Mix, 1 µL de primer from the mixture of each multiplex panel (each primer concentration was 10 mM) and 1 µL of DNA. We used negative (water) and positive (sample with a known allelic size) controls to detect potential contamination and confirming the allele sizes, respectively. PCR conditions followed (Suárez et al., 2009). All PCR products were sized with an ABI PRISM 3700 capillary sequencer analyser using the GS 500 LIZ size standard, and the software GeneMarker (Soft Genetics, State College, PA, USA).

We evaluated and compared the genetic variability of the newly founded population in relation to the core population of Inagua throughout three different approaches. First, we measured genetic diversity differences between both populations using standard genetic statistics, including observed (H<sub>O</sub>) and expected heterozygosities (H<sub>E</sub>), and allelic richness (AR). In addition, we calculated the fixation index (FST) defined by Weir & Cockerham (1984), and inbreeding coefficient (F<sub>IS</sub>) with the program ARLEQUIN version 3.5.1.2 (Excoffier & Lischer, 2010). Comparisons between populations were performed by t-tests. Second, we estimated the effective population size (N<sub>e</sub>) of both the core and newly founded populations by means of a corrected version of Waples & Do's (2008) linkage disequilibrium method (Waples, 2023) using the software NeEstimator version 2.1 (Do et al., 2014). The program calculates confidence intervals performing both standard parametric chi-squared methods and the jackknife method of Waples & Do (2008). The latter one seems to reduce potential bias associated with the confidence intervals calculated with standard parametric chi-squared methods (Waples & Do, 2008). For this analysis, we selected the option of excluding singleton alleles to avoid using potential unrealistic alleles. N<sub>e</sub>/N ratios were calculated considering recent population estimates (N) at La Cumbre and Inagua (Carrascal *et al.*, 2022). Finally, we evaluated the average relatedness and inbreeding coefficients of Inagua and La Cumbre using the software COANCESTRY version 1.0.1.10 (Wang, 2011). The average relatedness provides information about the level of differentiation between both demes (*i.e.*, local populations) in relatedness coefficients. COANCESTRY uses seven different relatedness estimators that account for inbreeding through two likelihood methods (Wang, 2011). These results informed us about the inbreeding levels occurring in both groups. The program uses a bootstrapping approach to obtain the 95% confidence interval to detect whether the groups analysed (*i.e.*, Inagua *versus* La Cumbre) differed for each estimator.

#### Results

#### **Environmental data**

Average daily temperatures in the shade in Inagua and La Cumbre were highly correlated from 1st March to 30th September 2017 (Fig. 2a; P << 0.001 in a generalised least squares GLS model with autoregressive -p = 3— moving average -q = 3— process to account for temporal autocorrelation;  $R^2 = 0.947$ ). Mean daily temperature was, on average, 1.2°C higher in Inagua than in La Cumbre (intercept of the model, se = 0.531). This difference increased as the weather got warmer (regression slope of Inagua on La Cumbre temperatures = 1.051, se = 0.026), especially during peaks of heat waves when average temperature was c. 3.0°C hotter in Inagua than in La Cumbre (eight clear short periods of intense increase in air temperature, four of them with average temperatures above 30°C in Inagua).

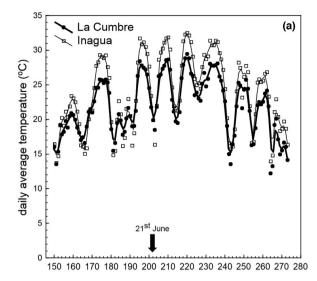
A similar pattern was observed for the amount of time per day when air temperature in the shade was above  $30^{\circ}$ C (Fig. 2b; P << 0.001 in a GLS model with autoregressive – p = 3– moving average –q = 3– process;  $R^2 = 0.937$ ). Average time per day with temperatures higher than  $30^{\circ}$ C was 39 minutes longer in Inagua than in La Cumbre (intercept of the GLS model, se = 10.1), although this difference increased as the weather got hotter (regression slope of Inagua on La Cumbre times with temperatures >30°C = 1.159, se = 0.036). There were 150-200 more minutes per day with temperatures higher than  $30^{\circ}$ C in Inagua than in La Cumbre during the five hottest heat waves.

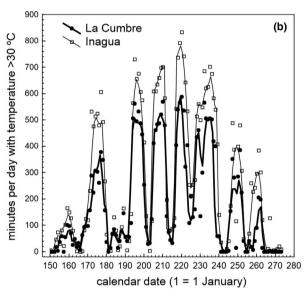
Therefore, the pine forest at La Cumbre located at a higher altitude than the pine forest at Inagua was cooler, especially during heat waves when the time per day with temperatures higher than  $30^{\circ}\text{C}$  was 150--200 minutes lower than in Inagua.

#### **Biometry**

# Tarsus length

There were significant differences in tarsus length between the two study areas (P = 0.013), with juveniles of Inagua





**Figure 2** Daily variation of average air temperature in the shade and time per day with temperatures higher than 30°C in La Cumbre and Inagua Strict Nature Reserve from 30 May to 30 September 2017. Curved lines define temporal smoothed patterns using locally weighted scatterplot smoothing (LOWESS).

(mean = 23.18 mm, se = 0.060, n = 65) being slightly larger than those of La Cumbre (22.99 mm, se = 0.065, n = 59). Males (23.17 mm, se = 0.068, n = 60) were also significantly (P = 0.044) larger than females (23.02 mm, se = 0.058, n = 64). The interaction Area × Sex was not significant (P = 0.194).

#### Wing length

Wing length was significantly (P << 0.001) larger in males (92.90 mm, se = 0.202, n = 60) than in females (86.80 mm, se = 0.220, n = 64), but did not differ between Inagua and La Cumbre (P = 0.133); the difference between males and

females remained unchanged in both study areas (P = 0.802 for the interaction term Area × Sex). The same biometric pattern was observed for tail length, with large, significant (P << 0.001), differences between males (75.38 mm, se = 0.203, n = 59) and females (70.79 mm, se = 0.200, n = 64), lack of significant differences between Inagua and La Cumbre (P = 0.465), and consistent inter-sexual differences in the two study areas (P = 0.694 for Area × Sex).

#### **Body mass**

Body mass (on a logarithmic scale) was positively related to tarsus length (slope = 0.027, se = 0.008, P = 0.001) across the two sexes and study areas (Fig. 3a). The adjusted mean of body mass was significantly larger (P << 0.001) in males than in females (Fig. 3b), and in La Cumbre than in Inagua (P = 0.005). Nevertheless, the interaction Area × Sex was also significant (P = 0.031), as the difference between the two study areas was only detected in females.

#### Feather asymmetry

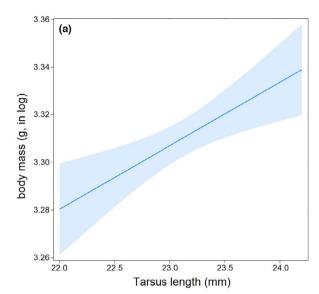
Length asymmetry of the right and left fifth rectrix was not significantly different between study areas (P = 0.401), sexes (P = 0.752), and their interaction (P = 0.790), with a very low average value of 0.202% (se = 0.025, P = 125).

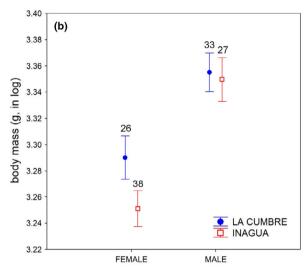
In summary, biometric differences (wing, tail and tarsus lengths) in juvenile chaffinches were mainly associated with sex, with higher values in males than in females, being minimal in relation to the two study areas (only tarsus length was slightly larger in males from Inagua than from La Cumbre). Asymmetry in tail feather length, as a measure of long-term body condition due to its sensitivity to environmental stress and response to differences in phenotypic quality, was very small with no differences between sexes or study areas. Body mass relative to tarsus length was larger in juvenile females from La Cumbre than from Inagua.

#### **Breeding success**

Actual nesting success in La Cumbre obtained by means of MARK estimation was 0.58 (se = 0.081; 95% confidence interval: 0.41–0.72, n = 61 nests). For the Inagua population, the average probability was 0.55 in 1991–2004 (se = 0.079; 95% confidence interval: 0.40–0.71, n = 86 nests; data from Rodríguez & Moreno, 2008). Thus, the breeding success showed a complete overlap according to their 95% confidence intervals in Inagua and La Cumbre.

Average probability of having two fledglings per successful breeding attempt was 0.53 the translocated population of La Cumbre (n = 47 successful nests; 95% confidence interval: 0.38–0.68). The average figure obtained by Rodríguez & Moreno (2008) for the Inagua population in 1991–2004 was 0.48, completely overlapped with that measured in La Cumbre (n = 63 successful nests; 95% confidence interval: 0.35–0.60). Average number of fledglings per successful breeding attempt in the first broods in La Cumbre was 1.53 (95% confidence interval: 1.38–1.68), very similar to the figure obtained by





**Figure 3** Partial influence plots of body mass (in log) variation related to a linear measurement of body size (tarsus length), sex and study areas. The figures of the Y-axes are adjusted means considering the effect of tarsus on body mass. Blue areas in panel A and the length of the whiskers around each data point in panel B are denoting 95% confidence intervals. Numbers above whiskers are sample sizes.

Rodríguez & Moreno (2008) for Inagua in 1991–2004 (1.45 fledglings; 95% confidence interval: 1.35–1.60).

Therefore, reproductive success and chick productivity in the first clutches were indistinguishable between the source population from Inagua and the translocated population from La Cumbre.

# **Genetic data**

Two out of nine microsatellite loci (Ftey20 and Ftey26) failed to amplify in more than half of individuals genotyped

and were removed for further analyses. Suárez *et al.* (2009) obtained a similar result with these microsatellites, which suggests that these two loci should be genotyped in different multiplex panels or alone. Genetic diversity indices obtained with the program ARLEQUIN are shown in Table 2, and they were similar for Inagua and La Cumbre. Differences between Inagua and La Cumbre were not significant for observed (t = -1.17, P = 0.26) and expected heterozygosity (t = 0.17, t = 0.87), allelic richness (t = 0.50, t = 0.62), or inbreeding coefficient (t = 1.56, t = 0.14) for the seven loci genotyped. The fixation index (t = 0.50, t = 0.018) are QUIN was very low but significant (t = 0.50, t = 0.001).

The contemporary effective population size was higher in the core population of Inagua than in the recently founded population at La Cumbre (Table 3). Confidence intervals provided by the jackknife method were more relaxed that the values calculated by the standard parametric chi-squared method.  $N_e/N$  ratios showed the highest values for La Cumbre (54.5/68 = 0.80) in relation to Inagua (118.7/362 = 0.33), with a global (*i.e.*, Inagua plus La Cumbre together) ratio for the species of 151.1/430 = 0.35.

The average relatedness calculated for the seven relatedness estimators provided low average values for all of them. La Cumbre obtained the highest values, and all estimators (TrioML, Wang, LynchLi, LynchLi, LynchRd, Ritland, QuellerGt, and DyadML) showed significant differences (P < 0.05) with Inagua in average relatedness (Table 4; Table S2). Finally, the two inbreeding estimates (*i.e.*, LynchRd and Ritland) were low (<0.1) in both demes, although the differences were significant (P < 0.05; Table 4).

# **Discussion**

We have evaluated the effectiveness of conservation actions performed in a recently founded population of an endangered island passerine using genetic parameters, breeding success, temperature data, and biometric variables related to juvenile size and body condition. The translocated population of the Gran Canaria blue chaffinch at La Cumbre, from juvenile individuals from captive breeding and captured from the source population of Inagua, seems to perform as viable in the short time, considering the similar values of biometric measurements, reproductive success, body condition, genetic diversity, and inbreeding of wild-born birds in both populations.

This translocation program had three distinctive features: the continuous release of juvenile birds year after year for ten consecutive years, two different origins for the released birds (juveniles coming from captive breeding and wild-caught juveniles from the source population), and the release of specimens in a locality less subject to extreme heat events. The continuous release of individuals for 10 years ensured that the initial population was reinforced during its settlement in the new locality. The soft-release of captive-bred individuals, with a very low tendency to show large movements (Delgado *et al.*, 2020), can guarantee that they settle in the same area they are released. The hard-release of wild-born juveniles from the source population can reinforce

**Table 2** Genetic diversity indices (mean  $\pm$  SD) obtained from seven polymorphic microsatellite loci in Inagua (core population) and La Cumbre (recently founded) of the Gran Canaria blue chaffinch

Population	n	AR	H <sub>o</sub>	H <sub>e</sub>	F <sub>IS</sub>
Inagua	57	9.26 ± 5.57	$0.72\pm0.10$	$0.76 \pm 0.12$	0.052 ± 0.17
La Cumbre	50	$7.70\pm4.94$	$0.78\pm0.11$	$0.75\pm0.10$	$-0.049\pm0.12$

n, sample size; AR, allelic richness; Ho, observed heterozygosity; H<sub>E</sub>, expected heterozygosity; F<sub>IS</sub>, inbreeding coefficient.

**Table 3** Effective population sizes ( $N_e$ ) estimated with an updated version of the linkage disequilibrium method (LD; Do *et al.*, 2014)

Population	n	LD	Parametric	Jackknife
Total	107	151.1	105.3–245.4	83.0–441.7
Inagua	57	118.7	69.8–299.8	62.9–452.8
La Cumbre	50	54.5	35.1-99.6	27.1-196.5

95% confidence intervals for  $N_e$  calculated with parametric and iackknife methods are also shown.

**Table 4** Summary statistics for seven estimators of average relatedness and two of inbreeding for the Inagua and La Cumbre populations (mean values, with variance in brackets) calculated with the program COANCESTRY (Wang, 2011)

	Inagua	La Cumbre	Significance
Relatedness			
TrioML	0.062 (0.010)	0.090 (0.016)	**
Wang	-0.014 (0.042)	0.053 (0.044)	**
LynchLi	0.003 (0.047)	0.055 (0.046)	**
LynchRd	-0.001 (0.019)	0.014 (0.026)	**
Ritland	-0.005 (0.021)	0.008 (0.015)	**
QuellerGt	0.001 (0.037)	0.019 (0.043)	**
DyadML	0.077 (0.015)	0.106 (0.022)	**
Inbreeding			
LynchRd	0.093 (0.052)	-0.015 (0.027)	**
Ritland	0.136 (0.131)	0.011 (0.013)	**

Significance (\*\*) and non-significance (ns) pairwise comparisons at 95% confidence level are also shown. TrioML: Triadic likelihood estimator (Wang, 2007). Wang: Wang's estimator (Wang, 2002). LynchLi: A moment estimator described in Lynch (1988) and Li, Weeks, & Chakravarti (1993) LynchRd: A moment estimator of pairwise relatedness described in Lynch & Ritland (1999). Ritland: A moment estimator described in Ritland (1996). QuellerGt: A moment estimator described in Queller & Goodnight (1989). DyadML: Dyadic likelihood estimator (Milligan, 2003).

the connectivity between the core and translocated populations (93% of those birds were detected in Inagua at least 200 days latter). The newly founded population was established in an area located at a higher altitude and less affected by the deleterious consequences of global warming, especially during the frequent heat waves that occur during the breeding season.

In the context of avian translocation programs, the feasibility of such initiatives hinges on identifying suitable habitats that support the physical well-being and reproductive

success of relocated species, ensuring their survival through natural reproduction (Gallerani et al., 2023). Additionally, the proximity of the translocation site to the source population is crucial, not only for potentially establishing genetic connections but also for effectively extending the species' range. This was exemplified by the translocated population of the Gran Canaria blue chaffinch at La Cumbre, which found an appropriate habitat to thrive in the wild, reproducing with similar levels of success and body condition to those measured in the source population at Inagua. Our study offers valuable insights for the endangered Azores bullfinch (Pyrrhula murina). This species' breeding population could increase significantly (by 19% or 27%) under realistic forest management scenarios, but expansion beyond its current restricted area remains ineffective due to local forestry dynamics and plant invasions (Bastos et al., 2012). Exotic tree species, particularly Clethra arborea, pose a significant threat to the Azores bullfinch population, with its invasion of native forests being the most significant environmental stochasticity factor (Ramos, 1996). The removal of invasive alien species in laurel forest restoration could increase native food resources for this critically endangered species, with potential short-term benefits from native plant re-establishment (Ceia et al., 2010). Therefore, in situations like that of the Azores bullfinch, translocation programs could be implemented if, and only if, there is a favourable habitat for the species' survival and reproduction in the wild, and a possibility of connecting with the source population.

#### Climatic data

Climate change has been linked to an increase in the frequency and severity of extreme weather events, such as storms, droughts, and heatwaves (IPCC, 2022). These events can directly impact bird populations by altering the timing of breeding, causing chick mortality, decreasing the body sizes of fledglings, and forcing parents to work harder to feed their young (Carey, 2009; Shipley et al., 2020). Or indirectly through food resource scarcity and its influence on the mismatch between the timing of peak food supply and nestling demand (e.g., Thomas et al., 2001; Sanz et al., 2003; Julliard, Jiguet, & Couvet, 2004; Carey, 2009). Scridel et al. (2018) propose that birds whose breeding distributions are largely restricted to mountains are likely to be more negatively impacted than other species. Areas at higher altitudes may be advantageous for adults and young birds during summer when habitats at lower elevations are hotter and dryer (e.g., Wiegardt et al., 2017; Saracco et al., 2018). The lower average temperature of 1.2°C, and three hours a day

n, sample size.

less with temperatures below 30°C, in La Cumbre than in Inagua will certainly contribute to reducing the deleterious effects of global warming on this incipient translocated population. It is plausible to speculate that these birds will increase their short-term viability, because metabolic rate and evaporative water loss steadily increase above 30°C in many passerines (e.g., Bicudo et al., 2010). The occurrence of the blue chaffinch in La Cumbre and Inagua is expected to counteract the adverse effects of global warming. Climatic forecasts suggest a temperature increase in the Canary Islands over the next 75 years (Expósito et al., 2015), with both Tenerife and Gran Canaria islands recording a rate of 0.17°C/decade since 1970 (Luque et al., 2014). Consequently, the recent establishment and reinforcement of the blue chaffinch population in La Cumbre, in conjunction with its proximity to Inagua and the extensive altitudinal range of pine forests in both areas, could enhance the overall viability of the species, especially when confronted with global warming and critical events such as forest fires.

#### **Biometric data**

Biometric measurements, such as wing, tail, or asymmetry of feathers serve as non-invasive tools for inferring and monitoring the variation in body size and quality of birds during the breeding season (Ricklefs, 1973; Tjørve & Tjørve, 2010). None of these measurements of juvenile Gran Canaria blue chaffinches differed for five consecutive years between the newly funded translocated population of La Cumbre and that of Inagua. Only tarsus length was slightly greater in juveniles from Inagua than from La Cumbre (0.82% longer). Nevertheless, mean of body mass, adjusted by body size, was significantly larger in La Cumbre than in Inagua, suggesting that juvenile blue finches from La Cumbre had better body condition at the end of the summer than those from Inagua. These slight differences could be attributed to the less stressful thermal conditions at La Cumbre, with potential effects on thermoregulation and water balance of birds. Therefore, it can be stated that the body size and condition of juveniles has been similar in both populations.

# Genetic data

From a genetic point of view, we did not find evidences that La Cumbre is an eroded population in comparison with Inagua. In fact, La Cumbre showed lower inbreeding values and higher observed heterozygosity than Inagua. The average relatedness calculated for the seven relatedness estimators provided low values for both populations, although they were significant for all of them. La Cumbre showed the highest relatedness values, and this result could be explained by the limited genetic management performed with the released birds. That is, birds caught in Inagua, and released immediately in La Cumbre, were not genetically selected, nor did the captive breeding program carry out a systematic evaluation of the genetic relatedness of the birds paired. We encourage managers to perform a strict genetic selection of birds released in future translocations. Therefore, the slightly

higher relatedness values obtained in La Cumbre in relation to Inagua could be an expected result. However, it had no negative effects (*i.e.*, higher inbreeding values, which could have driven to loss of heterozygosity and the accumulation of deleterious alleles) for the newly founded population. The low genetic differentiation recorded between La Cumbre and Inagua reflects weak structuring, which is a direct consequence of the recent origin of La Cumbre population.

The small effective population size observed at La Cumbre  $(N_e = 54.5)$  was expected due to the limited area of this Canary pine forest patch. Despite the fact that this value falls in the limit of standard recommendations of minimum viable sizes (e.g., the classic  $N_e = 50-500$  for short and long term, Franklin, 1980), such standard parameters must not be directly related with extinction risk, but with thresholds of standing genetic variation (i.e., the genetic variation that the species harbour) to be maintained over time (Flather et al., 2011; Alves et al., 2023). In fact, extensive runs of homozygosity with subsequent loss of genetic diversity and low effective population sizes are not rare scenarios in small but persistent island avian populations (Duntsch et al., 2023; Martin et al., 2023). The N<sub>e</sub>/N ratio was higher at La Cumbre (0.80) than Inagua (0.33), which is a likely consequence of being a small population with low inbreeding values (Caballero & Hill, 1992). These values fall within those obtained with other animals where N<sub>e</sub>/N is expected to be less than or equal to 0.5 (the global value for the species was 0.35), but there are cases with values above 0.8 (Nunney, 1993; Nunney & Elam, 1994; Hoban et al., 2020). N<sub>e</sub> for the translocated population of the Gran Canaria blue chaffinch was similar to the estimated figure for the newly founded Seychelles warbler (Acrocephalus sechellensis) populations in the archipelago of Seychelles, after being successfully translocated on four islands (Wright et al., 2014). Nevertheless, our genetic parameters at La Cumbre were higher than those recorded with the Seychelles warbler (Wright et al., 2014), which suggests that Gran Canaria blue chaffinch could be in a better situation to face future environmental challenges than the Seychelles warbler, especially under the current scenario of connectivity recorded between Inagua and La Cumbre.

Conservation biology is a relatively young scientific discipline that arose with the aim of providing solutions to the species, communities and ecosystems severely modified by human actions (Soulé, 1985). Local, regional, national, and international stakeholders and policy-makers are continually performing conservation actions with the final goal of halting the loss of biodiversity. Determining the effectiveness of the conservation planning actions is difficult when not all necessary assessment parameters are being measured and monitored frequently and regularly. However, assessing the effectiveness of conservation actions should be a priority because such evaluations will help to establish whether they were suitable to the target established or if they need to be modified (Sutherland et al., 2021). With the present study, we have conducted a multiple trait assessment of the viability of a newly founded population in the Gran Canaria blue chaffinch, an endemic and endangered forest passerine with a restrictive distribution within Gran Canaria.

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Overall, our genetic results reveal that La Cumbre shows genetic parameters similar to the core source of Inagua, which contrasts with the lower genetic diversity and higher inbreeding values expected due to the small size of the newly founded population (Illera, Emerson, & Richardson, 2007; Spurgin et al., 2014). In addition, the biometric results, together with those showing similar reproductive success in both populations, and a sustained growth of the population at La Cumbre from two breeding pairs in 2010 to 29 in 2019 (Delgado & Carrascal, 2021), suggest that the translocation program to a new locality situated at a minimum distance of 2 km from the nearest border of the source population can be deemed successful ten years after it was initiated. The approach and results of this translocation program with the Gran Canaria blue chaffinch, which combined soft and hard-release and an annual monitoring of the source and newly founded populations, provide insights on the effectiveness of the actions performed, and may illuminate the conservation planning for other endangered upland forest bird species inhabiting island ecosystems, especially those jeopardised by the effect of global warming.

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#### **Author contributions**

Conceptualization: LMC. Data curation: AD, DT, JCI, LMC. Formal analyses: JCI, LMC. Writing: JCI, LMC. Review and editing: AD, DT, JCI, LMC. Funding Acquisition: JCI, LMC.

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# **Supporting information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** Gran Canaria blue chaffinch individuals used in the present study for each type of analyses. Sex: 1 (male), 2 (female). Wing chord length (mm), body mass (weight, g), tarsus-metatarsus length (mm), tail length (mm) and length of the right and left fifth rectrices (left \_TF and right\_TF, respectively, mm). na: not available data.

**Table S2.** Relatedness values estimated from the seven methods available in the program Coancestry (Wang, 2011), for the 57 and 50 individuals analysed in Inagua and La Cumbre, respectively. Rows show the estimates per dyad.